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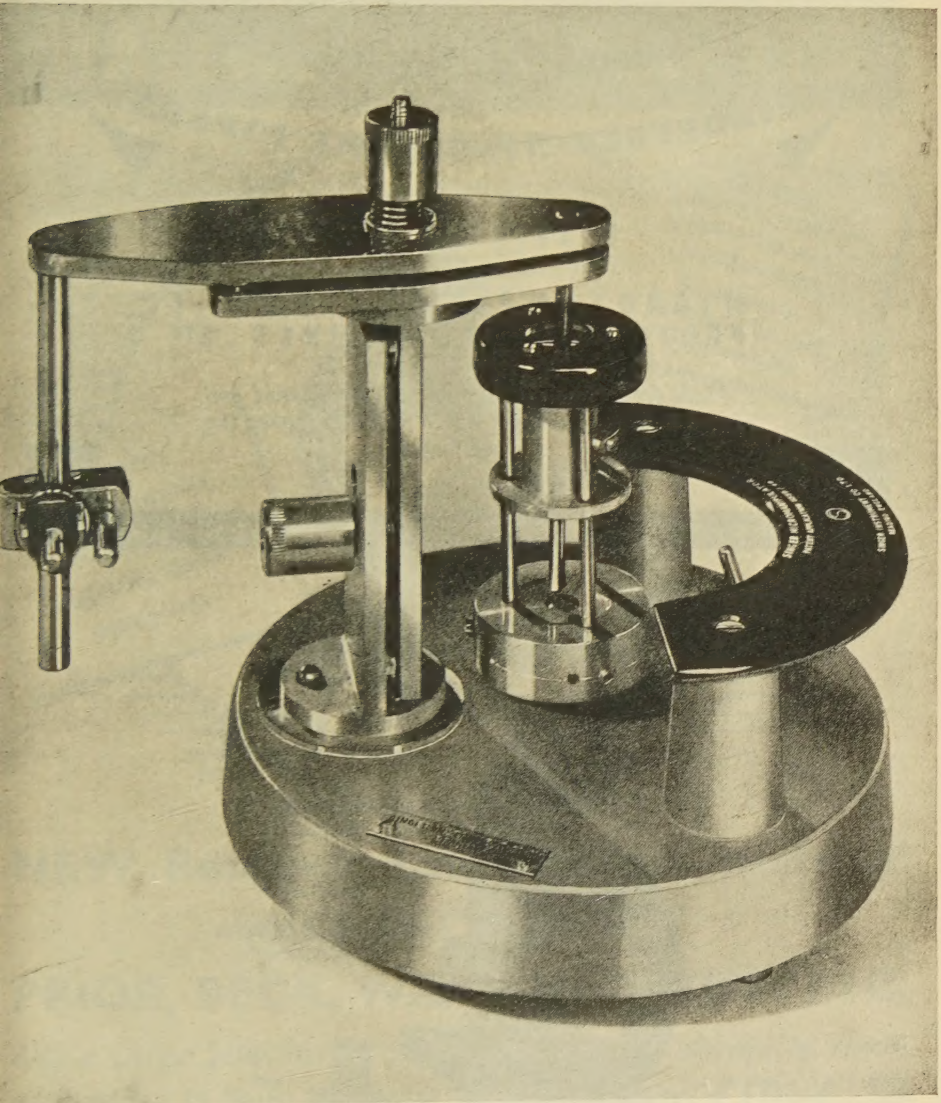

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Cyclic Changes in Polysaccharides of the Papilla of the Hair Follicle

By WILLIAM MONTAGNA, HERMAN B. CHASE, JANET D. MALONE, AND HELEN P. MELARAGNO

(*From Brown University, Providence, Rhode Island, U.S.A.*)

Summary

In the skin of the mouse the dermal papilla becomes metachromatic and PAS-reactive on the 4th day after the initiation of growth in the hair follicle. This is a stage of follicle development which immediately precedes hair proliferation. Both metachromasia and PAS reaction remain intense until about 18 days, just preceding catagen, or club formation, when they disappear abruptly. During telogen, or the resting stage, the papilla is neither metachromatic nor PAS-reactive. The metachromasia and the PAS reaction of the dermal papilla during the period of active hair proliferation indicate the presence of at least two polysaccharides which are not detectable during the remainder of the cycle.

Introduction

IN the skin of the mouse we find that the dermal papilla of growing hair follicles stains metachromatically with toluidin blue, but in follicles with resting hairs, the papilla is not metachromatic. Similarly, when treated with the periodic acid/Schiff (PAS) reagent method for 1, 2 glycol groups, the dermal papilla of active hair follicles is PAS-reactive but that in resting follicles is not. Presumably, then, one or two substances are present in the active follicle but absent in the resting one. Sylvén (1950) first called attention to the rise and decline of metachromasia in the hair follicle of the rat, during growth and rest, and Johnson *et al.* (1945), also in the rat, have demonstrated alkaline phosphatase in the dermal papilla of the active, but not the inactive hair follicle.

It is generally believed that the dermal papilla of hair follicles plays an important role in the normal growth and differentiation of hair and hair follicles. During the phases of hair growth, anagen, catagen, and telogen (Dry, 1926), the papilla undergoes striking morphological changes, and these changes have been described in detail in the mouse by Chase *et al.* (1951). Little is known, however, of the corresponding physiological changes in the papilla and of their relation to the induction of growth and maintainance of hair follicles.

Material and Methods

Twelve C_3H mice approximately 80 days old were used. An area of skin approximately 2 cm.^{2} of the dorsum of each mouse was plucked of its club

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hairs. Since plucking of hair initiates growth in a resting (telogen) follicle, biopsy specimens taken at specific intervals after plucking gave us the precise growth time-sequence of the follicles. Biopsy specimens were taken from the plucked area at 1, 2, 3, 4, 5, 6, 12, 18, and 24 days. All tissues were fixed in Zenker-formal, embedded in paraffin, and sectioned at 5 μ. For the demonstration of metachromasia, sections were stained 5 minutes in 1/2,000 solution of toluidin blue buffered to pH 4·0, 5·0, and 6·0. The slides were then rinsed quickly in distilled water, dehydrated 5 minutes each in three changes of tertiary butyl alcohol, then 2 minutes in absolute ethyl alcohol, cleared in toluene, and mounted in piccolyte dissolved in toluene. The 1, 2, glycol groups were demonstrated by treating sections of Helly-fixed tissues with the McManus and Cason (1950) periodic acid/Schiff reagent technique. Deparaffinized sections were run down to water and placed for 10 minutes in 0·5 per cent. aqueous periodic acid warmed to 37° C. After a brief rinse the slides were placed in the Schiff reagent for 20 minutes, rinsed for 5 minutes in each of three changes of sulphurous acid, washed, counterstained with haematoxylin, dehydrated, and mounted in piccolyte. Control slides were treated with saliva before the application of the test. The reactive substance in the dermal papilla of growing follicles is not removed by saliva and is not glycogen. It will simply be referred to in this paper as PAS-reactive substance.

Observations

The phase of active growth in the hair follicle is called anagen, the short phase of transition and club formation is called catagen, and the resting phase is called telogen (Dry, 1926). Chase *et al.* (1951) observe that the anagen stage is characterized by six readily recognized substages. The following observations on metachromasia and periodic acid/Schiff reactive substances in the papilla are correlated with these six critical substages of anagen and with catagen and telogen. Plucking of club hairs from follicles initiates growth of new hairs in these follicles. It is possible to observe all of the stages of the hair growth cycle within a period of 20 to 24 days after plucking. Thus, in this paper the number of days in the development of the hair follicles refers to the number of days since the club hairs were plucked from the follicles. For clarity and brevity the descriptions of hair follicles will follow closely those of Chase *et al.* (1951).

In the resting or telogen follicle, the rounded dermal papilla lies just beneath the germ plate. The papilla cells are small, each having a dense nucleus and a narrow halo of cytoplasm. With toluidin blue, only the nuclei stain clearly, the cytoplasm remaining colourless. After the tissues are treated with PAS the cells of the papilla remain colourless.

In skin one and two days after plucking the cells in the germ plate are in mitotic division and the plate is enlarging. In this phase of growth, 'anagen I', the papilla cells have enlarged in diameter, the nucleus is less dense, and the cytoplasm is more abundant. The cytoplasm of the papilla cells and the intercellular matrix are still neither metachromatic nor PAS-reactive.

At 3 days after plucking, 'anagen II', there is a downgrowth of the germ plate around the upper end of the ball of papilla cells. The inverted cup of hair germ cells thus formed is the beginning of the bulb with its enclosed 'papilla cavity'. The papilla cells have become still larger, showing a large nucleus and a moderate amount of clear cytoplasm. The cells are not metachromatic. After treatment with PAS the intercellular matrix is sharply reactive and appears as a delicate line which outlines each papilla cell, the cytoplasm of which continues to remain non-reactive.

On the fourth day after plucking, 'anagen III', the hair bulb completely encloses the papilla. The large papilla cavity is broad at the upper end and middle but constricted at the base. The papilla cells are very large. After staining with toluidin blue buffered to pH 4·0 to pH 6·0, the cytoplasm shows a very delicate metachromatic stippling. The fine lines of intercellular matrix show sharper metachromasia. At this stage the intercellular matrix becomes also conspicuously PAS-reactive.

On the fifth and sixth days after plucking, 'anagen IV', the hair bulb has formed a plumule of hair shaft and internal root sheath extending to the level of the sebaceous gland. The papilla is very large and is becoming increasingly elongated. The large papilla cells are riddled with colourless vacuoles but the cytoplasm and intercellular matrix are richly metachromatic. The papilla cells also contain lightly PAS-reactive granules and are surrounded by a brightly reactive matrix. As the papilla becomes increasingly elongated and its cells vacuolated, both metachromatic and PAS-reactive substances become progressively abundant until the twelfth day after plucking, at the middle of 'anagen VI'. Metachromasia and PAS-reactive substances remain in evidence until about the eighteenth day when they both disappear abruptly. This is the end of 'anagen VI' and the beginning of catagen.

On the twenty-fourth day after plucking, in the telogen stage, the hair follicle is again in the resting phase and a ball of small papilla cells lies under the germ plate. The papilla cells are neither metachromatic nor PAS-reactive.

Discussion

The studies of Sylvén (1950), Holmgren (1940), Dempsey *et al.* (1947), and Wislocki and Sognnaes (1950) present evidence that metachromasia in tissues reveals the presence of acid polysaccharides. It is likely also, at least as far as mast cell granules are concerned, that metachromatic staining with toluidin blue is due to an electrostatic linkage of sulphate groups with dye-ions (Landsmeer, 1951).

Materials which colour with the Schiff reagent after oxidation with periodic acid appear to be carbohydrates containing the 1, 2 glycol groups (Leblond, 1950, McManus and Cason, 1950), chiefly mucopolysaccharides. Lillie (1950), however, believes that the reaction simply shows aldehydes formed from 1, 2 glycols without inference as to the mucopolysaccharide structure.

Although the distribution of metachromatic and PAS-reactive substances in the papilla appear superimposable, it is probable that different substances

are being shown. Some tissue elements such as mast cell granules are strongly metachromatic, but stain weakly or not at all with the PAS technique; conversely, basement membranes and cuticular borders are PAS-reactive but not metachromatic. Metachromasia in skin seems to reveal acid polysaccharides, and probably chondroitin sulphate (Pearse and Watson, 1949; Meyer and Rapport, 1951). Since chondroitin sulphates are only mildly oxidized with periodic acid (Meyer and Odier, 1946), the strongly PAS-reactive substance in the dermal papilla may represent something in addition to an acid polysaccharide.

Sylvén (1950) has observed the cyclic increases and decreases of metachromasia in the growing and resting hair follicle of the rat. Montagna *et al.* (1951 *a*, *b*) have reported abundant metachromasia, and strong PAS-activity in the papilla of growing hair follicles in the human mons pubis. Sulphated acid polysaccharides (metachromatic), 1, 2 glycol groups (PAS reactive), and alkaline phosphatase (Johnson *et al.*, 1945), are present together in the dermal papilla of the growing hair follicle, but not in that of the resting follicle. Finding alkaline phosphatase and acid polysaccharides together is of particular interest since Sylvén (1947) reported that in endochondral ossification, chondroitin sulphate disappears in the cartilage matrix as alkaline phosphatase increases.

There is some similarity between the relationship of the dermal papilla to the hair bulb and that of the pulp to the tooth. Wislocki and Sognnaes (1950) find that the pulp of growing teeth is strongly metachromatic and rich in alkaline phosphatase activity. In adult teeth which have completed their growth, metachromasia is greatly diminished or completely absent, but phosphatase activity, unlike that of the resting papilla, remains strong.

These substances, whatever may be their exact nature, are abundantly present in the dermal papilla only, during most of the growth of the hair follicle and they may play a role in the process of hair growth. To be more precise, they appear by 'anagen III', 4 days after plucking at the stage of follicle development which immediately precedes hair proliferation. They disappear abruptly at the end of 'anagen VI', just preceding catagen, when the bulb is still fully intact. At this time, however, mitosis in the matrix cells will cease to occur and the transformation from catagen to telogen, or resting stage, will take place. The association between the presence of these substances and the period of active hair proliferation is evident.

Acknowledgement. This work was supported in part by grants from the United States Public Health Service and by a grant from the American Cancer Society, recommended by the Committee on Growth of the National Research Council.

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Phosphomonoesterase Content and Localization in the Meso- and Metanephros of the Chick Embryo

By L. C. U. JUNQUEIRA

(*From the Laboratório de Histologia e Embriologia, Faculdade de Medicina, Universidade de S. Paulo, Brazil, and Department of Zoology, Washington University, St. Louis, Mo., U.S.A.*)

With two plates (figs. 6 and 7)

Summary

Chemical determinations were made of the alkaline and acid phosphatase content of meso- and metanephros of chick embryos between 4 days of incubation and 6 days after hatching. Histochemical localization of alkaline phosphatase and of ribonuclease-removable basiphilia was also studied in these organs.

A study of the data obtained suggest the following conclusions:

1. The acid and alkaline phosphatase activity of the mesonephros increases from the 4th to the 16th day, decreasing from then onwards.

2. The acid and alkaline phosphatase activity of the metanephros increases from the 11th to the 16th day, decreasing slowly to the 20th day. A rapid rise is observed after hatching.

3. The alkaline phosphatase activity that is diffusely located in the cells of recently formed secretory tubules of the meso- and metanephros polarizes to the brush border zone later on.

4. Ribonuclease-removable basiphilia, abundant in the mesonephric tubules up to the 6th day of incubation, decreases from then onwards.

5. The metanephric blastema and tubules present a high concentration of ribonuclease-removable basiphilia up to the 13th day. From then on its content decreases in the whole organ with the exception of certain groups of cells still in differentiation.

The possible correlation between these findings and meso- and metanephros differentiation and function is discussed.

Introduction

ALTHOUGH several papers have been published on the chick embryo mesonephros, the time of beginning and end of its function still remains in doubt. Considerably fewer data can be found in the literature concerning the metanephros.

In an attempt to investigate this problem a study of the acid and alkaline phosphatase content and alkaline phosphatase localization was undertaken in the meso- and metanephros of chick embryos. Previous results in this field were reported by Moog (1944), who studied the acid and alkaline phosphatase distribution in chick embryos up to the 8th day of incubation. Consequently her data are restricted to the early development of the mesonephros.

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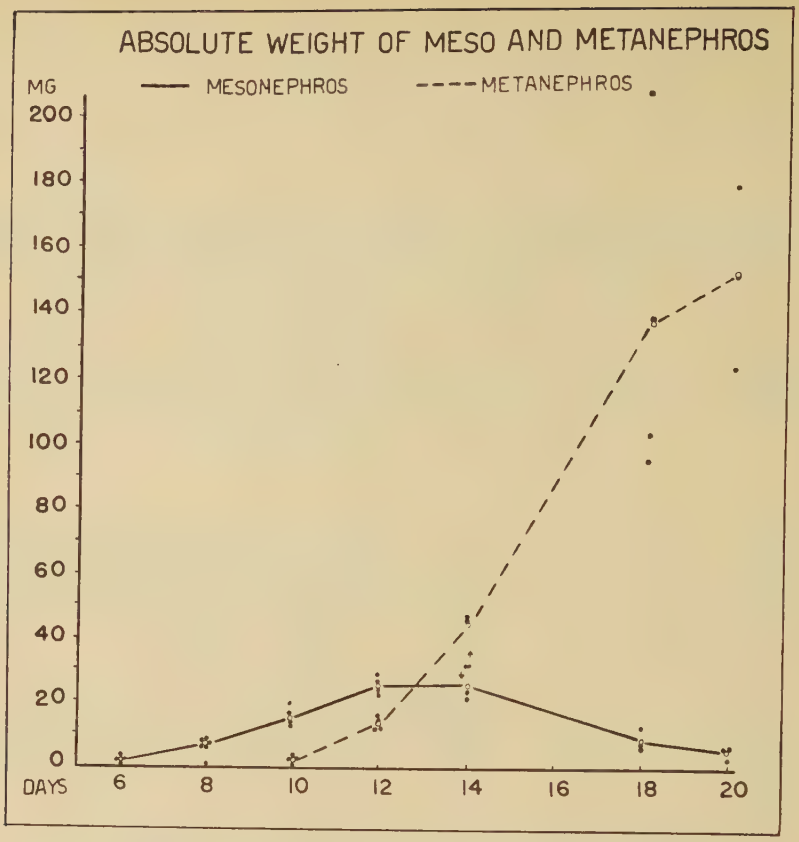

FIG. 1. Variation in the absolute weight of chick meso- and metanephros.

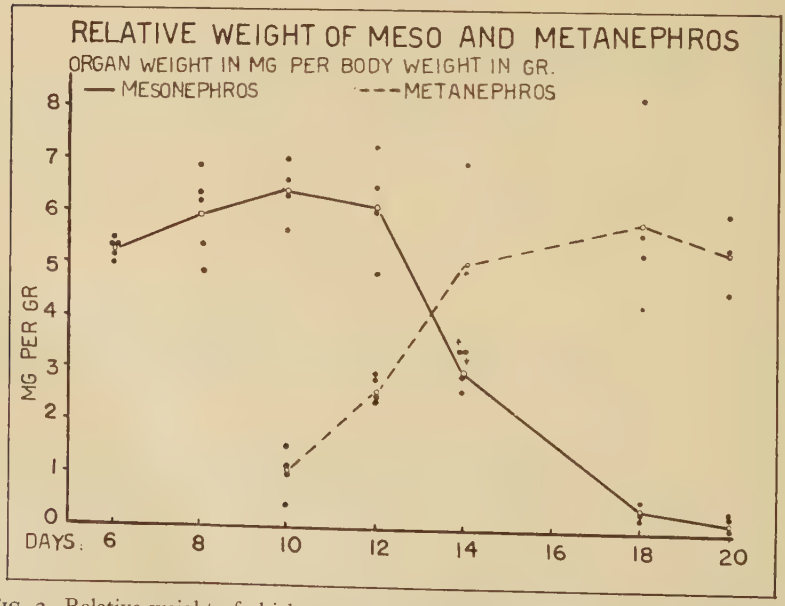

FIG. 2. Relative weight of chick meso- and metanephros. Observe substitution of meso- by metanephros around the 13th day.

Brachet's toluidin blue ribonuclease technique was applied to sections to study the distribution of the ribonuclease-removable basiphilic substance in embryonic chick kidneys in an attempt to correlate this substance with the differentiation of these organs.

Material and Methods

Eggs of New Hampshire Red chickens were incubated for desired periods at 39° C. The weight curves were made from meso- and metanephros of chicks of several ages fixed in Zenker-formalin, washed in water for 24 hours, and stored in 70 per cent. alcohol. No fresh weight determinations could be made owing to the difficulty of dissecting the kidneys in young stages. The histochemical localization of alkaline phosphatase was made using Gomori's (1939) method on iced 80 per cent. alcohol-fixed material. The sites of alkaline phosphatase activity appear stained by this technique in black or dark brown. The shrunken appearance of the tissues is due to the fixation. Chick embryos ranging from 3 to 20 days' incubation were used, and in some instances chicks were used 6 days after hatching. A minimum of four embryos was used for each age studied.

Brachet's ribonuclease method was used as described by Stowell and Zorzoli (1947).

Quantitative determinations of acid and alkaline phosphatase were made by the method described by Binkley, Shank, and Hoagland (1944). Since often only small amounts of tissue were available, this method was adapted for our purposes by using half of the amounts of substrate and reagents described in the original paper. This procedure permitted the use of 5 mg. of tissue (wet weight) for each determination. Readings were made in a Klett colorimeter.

The results presented are the averages of at least four determinations of each age. Incubation time was 30 minutes for the alkaline phosphatase and 60 minutes for the acid phosphatase. In addition, slides of Zenker-formalin material were stained with haematoxylin and eosin and compared with those used for histochemical studies.

Results

Results obtained by weighing the meso- and metanephros are summarized in figs. 1, 2, and 3. Fig. 1 shows that the absolute weight of the mesonephros increases gradually to the 14th day and from then on declines slowly. The relative weight curves (fig. 2) show that on approximately the 10th day the mesonephros stops growing proportionally to the body weight, while the metanephros grows very rapidly until the 14th day, its growth becoming from then on roughly proportional to the embryo's body weight.

Fig. 3 indicates that the relative total kidney weight grows gradually to a maximum at 12 days and decreases from then on.

Biochemical results: Results obtained in chemical determinations of phosphatases are summarized in Table 1 and in figs. 4 and 5. These figures suggest

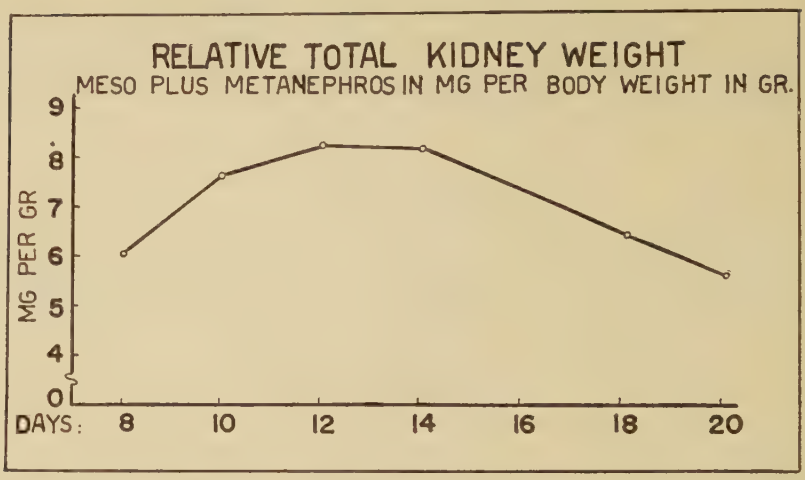

FIG. 3. Relative total kidney weight. Observe relatively small variations during the period observed as compared to changes in absolute weight.

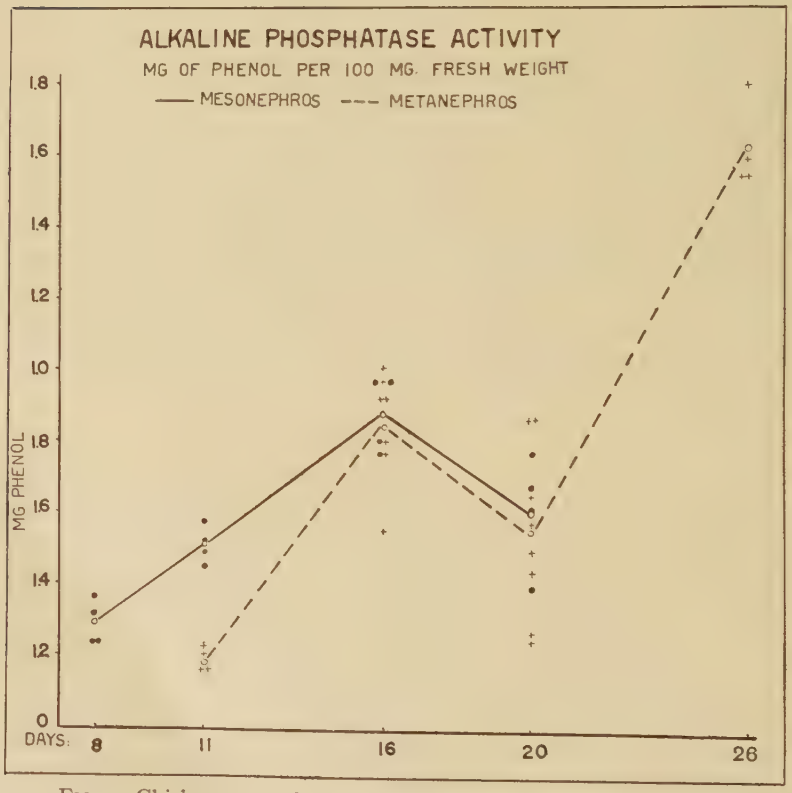

FIG. 4. Chick meso- and metanephros alkaline phosphatase activity.

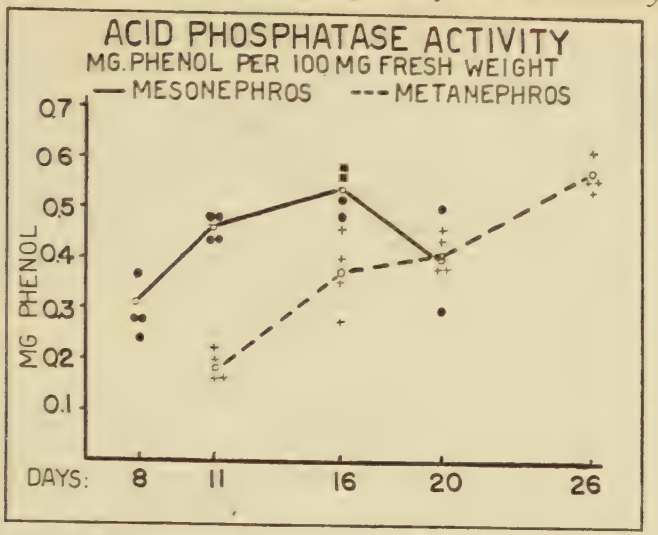

FIG. 5. Chick meso- and metanephros acid phosphatase activity.

TABLE 1. *Phosphatase Activity of Meso- and Metanephros**

	Mesonephros		*Metanephros*	
Days	*Acid phosphatase*	*Alkaline phosphatase*	*Acid phosphatase*	*Alkaline phosphatase*
8	0·28 $\bar{x}$ = 0·315 0·28 s = 0·041 0·34 cv = 13·0% 0·36	0·24 $\bar{x}$ = 0·290 0·36 s = 0·060 0·32 cv = 20·7% 0·24		
11	0·44 $\bar{x}$ = 0·460 0·48 s = 0·023 0·48 cv = 5·0% 0·44	0·44 $\bar{x}$ = 0·495 0·48 s = 0·050 0·56 cv = 12·1% 0·50	0·22 $\bar{x}$ = 0·185 0·16 s = 0·030 0·20 cv = 16·2% 0·16	0·20 $\bar{x}$ = 0·185 0·16 s = 0·030 0·22 cv = 16·2% 0·16
16	0·58 $\bar{x}$ = 0·535 0·52 s = 0·044 0·56 cv = 8·2% 0·48	0·80 $\bar{x}$ = 0·870 0·96 s = 0·150 0·96 cv = 12·1% 0·76	0·36 $\bar{x}$ = 0·375 0·46 s = 0·075 0·40 cv = 20·1% 0·28	0·96 $\bar{x}$ = 0·846 0·76 s = 0·152 1·00 cv = 17·9% 0·92 0·80 0·92 0·56
20	0·30 $\bar{x}$ = 0·400 0·40 s = 0·100 0·50 cv = 25·0%	0·62 $\bar{x}$ = 0·620 0·44 s = 0·163 0·76 cv = 26·3% 0·66	0·44 $\bar{x}$ = 0·415 0·38 s = 0·041 0·46 cv = 9·9% 0·38	0·26 $\bar{x}$ = 0·550 0·28 s = 0·223 0·44 cv = 40·5% 0·58 0·84 0·50 0·66 0·84
26			0·56 $\bar{x}$ = 0·570 0·54 s = 0·034 0·62 cv = 5·9% 0·56	1·56 $\bar{x}$ = 1·630 1·60 s = 0·114 1·56 cv = 6·9% 1·80

* Expressed in mg. of phenol liberated by 100 mg. of organ (fresh weight).
Legend: $\bar{x}$ = mean. s = standard deviation. cv = variation coefficient.

a gradual increase of the acid and alkaline phosphatase content of the mesonephros to a peak about the 16th day and a more rapid decrease from then to the 20th day.

It is interesting to observe the increase of the acid and alkaline phosphatase content of the metanephros from the 11th to the 16th day and subsequent decrease to the 20th day followed by an increase after the hatching and consequent feeding of animals. Another point of interest is the fact shown in the figures that the changes in both phosphatases are rather alike for the same organ and that apparently the alkaline phosphatase content always presents more intense changes than the acid phosphatase.

Histochemical results: The morphology of the meso- and metanephros varies greatly according to the zone sectioned. This is due to the cranio-caudal gradient of differentiation which these organs present. Descriptions made here are from approximately the middle of the gland. In the 3-day embryos the mesonephros is still differentiating, and adjacent to the Wolffian duct we see an aggregation of differently shaped cells that stain darkly by Gomori's alkaline phosphatase method (fig. 6, A). This region will later give rise to the mesonephric tubules, as shown in fig. 6, B, where in a section of the caudal portion of the mesonephros of a 4-day embryo the tubules are being formed by the mesonephric blastema. It was observed that the newly formed secretory tubules have a strong alkaline phosphatase activity in the cytoplasm. In a section of a more cranial region (fig. 6, C), one can see the formed tubules and a

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FIG. 6 (plate). The photomicrographs represent sections treated by Gomori's alkaline phosphatase technique. The scales represent 50 μ.

A. Section of the mesonephric region of a 3-day chick embryo. Observe Wolffian duct with a strong reaction in the cell nuclei and the mesonephric blastema below it with dark-staining cells indicating high alkaline phosphatase activity. Six hours' incubation. ×160.

B. Section from a 4-day embryo. Observe strong diffuse alkaline phosphatase activity in newly formed tubules and blastema of the caudal region of mesonephros. Six hours' incubation. ×160.

C. Section from the same embryo in a more cranial region. There is a localization of alkaline phosphatase activity in the secretory tubules with a beginning of its polarization to the brush border region. Four hours' incubation. ×240.

D. Mesonephros and gonad of a 6-day embryo. Observe strong positive alkaline phosphatase reaction in the secretory tubules with no reaction in the cytoplasm of the cells of the excretory tubules and glomeruli. Five hours' incubation. ×40.

E. Mesonephros from an 8-day embryo. Observe greater amount of tubules and glomeruli when compared with the 6-day embryo. A strongly positive reaction may be observed in the Müllerian duct. Five hours' incubation. ×40.

F. Section of an embryo of the same age showing the metanephric blastema with a strongly positive reaction and the beginning of tubule formation. Two hours' incubation. ×40.

G. Meso- and metanephros of a 10-day embryo. Observe increase in the tubules formed by the metanephric blastema. The mesonephric tubules present an increase in alkaline phosphatase activity when compared with the 6- and 8-day embryos. Five hours' incubation. ×40.

H. Mesonephros of an embryo of the same age. An intense reaction with polarization towards the apical region is visible in the secretory tubules. Glomeruli and excretory tubules present practically no activity in the cytoplasm of their cells. Five hours' incubation. ×160.

I. Metanephros of an 11-day embryo. Observe abundance of tubules with an alkaline phosphatase reaction. The blastema tissue is still present and shows an intense reaction. Five hours' incubation. ×120.

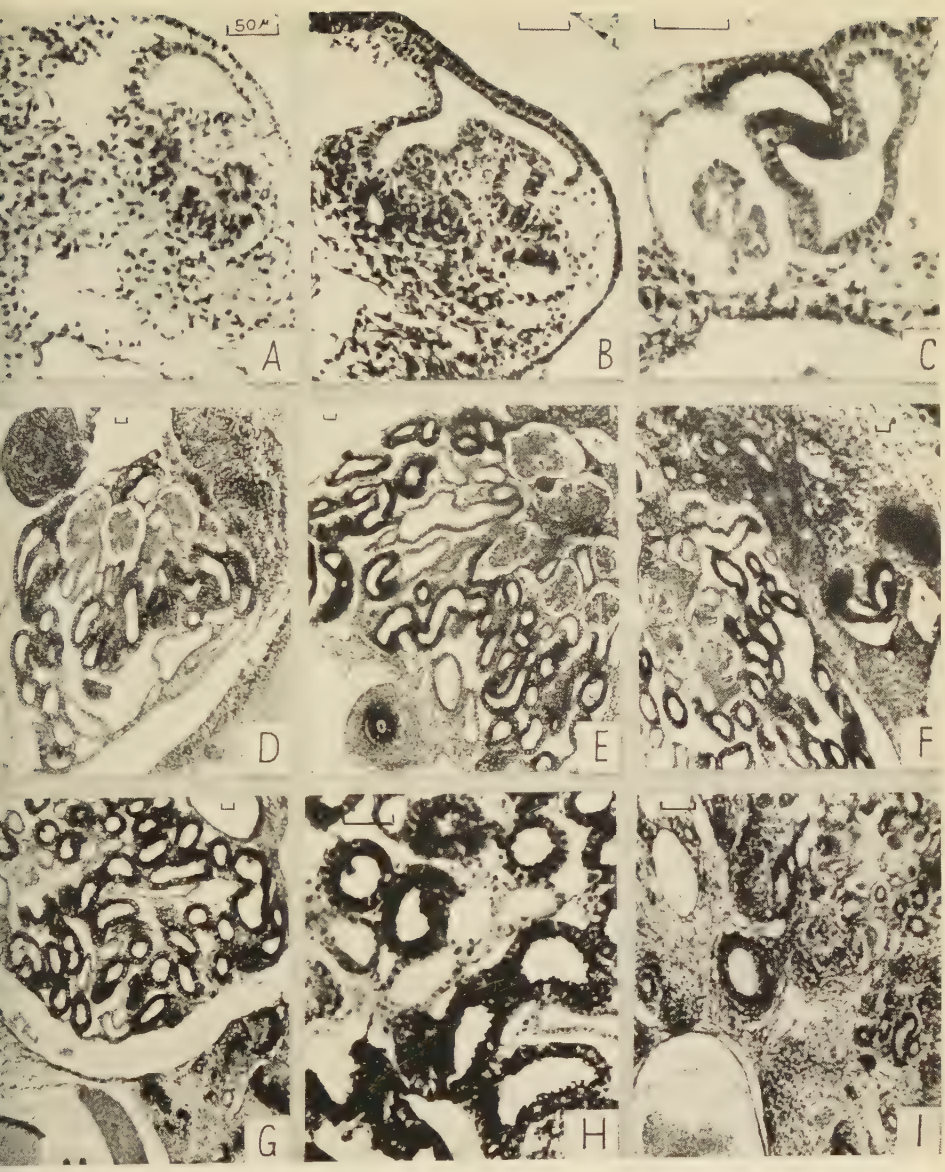

FIG. 6

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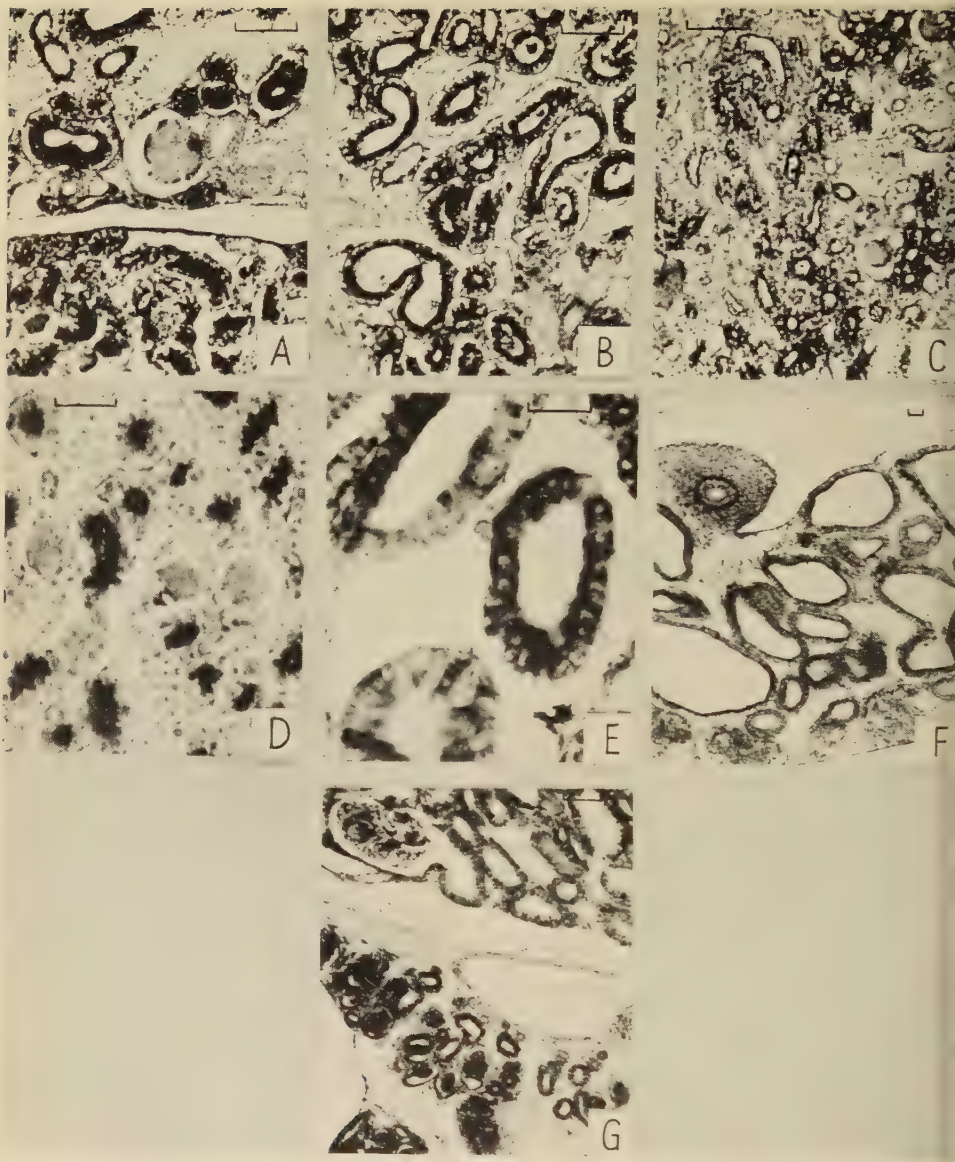

FIG. 7

L. C. U. JUNQUEIRA

difference in alkaline phosphatase activity between secretory and excretory tubules. Apparently, at this early age the secretory tubules already have a higher alkaline phosphatase activity than the excretory ducts. A careful examination of this figure will also show a beginning of polarization of the alkaline phosphatase activity to the brush border region. Glomeruli do not show alkaline phosphatase activity in sections of any of the embryos studied.

In the 6-day embryos there is a well-formed mesonephros in which the secretory and excretory tubules are easily distinguished by the intense alkaline phosphatase activity in the former and lack of activity in the latter. A polarization of alkaline phosphatase activity to the brush border region of the cell is quite evident in fig. 6, D.

The 8-day embryo presents a more highly developed mesonephros with more tubules and glomeruli than those previously described (fig. 6, E). Here one can see that the alkaline phosphatase activity has the same distribution as previously described. In fig. 6, F (also of 8 days) it is interesting to observe the metanephric blastema surrounding the metanephric tubules that are being formed. This blastema is composed of closely packed and variously shaped cells that stain darkly with Gomori's technique. This aspect is very striking if one compares these cells with adjacent mesenchyme cells that present practically no alkaline phosphatase activity. In sections of 10-day embryos (fig. 6, G and H) a picture very similar to that already described may be observed, although the secretory mesonephric tubules present a more intense alkaline phosphatase activity than in the 8-day embryo. The metanephros is more advanced in development and a greater quantity of newly formed tubules and blastema may be seen. These tubules that are being formed present a high concentration of alkaline phosphatase in their cytoplasm. The

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FIG. 7 (plate). A–D represent sections treated by Gomori's alkaline phosphatase technique; E–G stained with toluidine blue. The scales represent 50 μ.

A. Section of a 15-day embryo. Mesonephros in the upper part presenting a very strong reaction in the secretory tubules. The metanephros presents a distinct polarization of a strong alkaline phosphatase activity in the apical region of the secretory tubule cells. Five hours' incubation. ×200.

B. Mesonephros of a 20-day embryo. Observe disintegration of the mesonephric ducts with shedding of particles presenting alkaline phosphatase activity. Five hours' incubation. ×200.

C. Section from a metanephros of a 20-day embryo. Observe a distinctly diminished alkaline phosphatase activity with a narrow band of activity in the brush border zone. This is particularly evident if we compare it with A or D. ×200.

D. Metanephros of a 6-day-old chick. Observe intense and polarized activity in the secretory tubule cells. Glomeruli and excretory ducts present practically no activity in the cytoplasm of its cells. Five hours' incubation. ×200.

E. Mesonephros of a 5-day embryo presenting its tubules darkly stained by toluidin blue. ×200.

F. Section of an 8-day embryo mesonephros. Observe lighter staining of the tubules. The Müllerian duct cells present their basal region rich in R.R.B. ×40.

G. Meso- and metanephros of an 11-day-old chick embryo. Compare the dark staining of the metanephric blastema and tubules (in the lower part) with the lightly stained mesonephros in the upper part). ×120.

metanephros of the 11-day embryo is still more advanced in development and has several thin-walled tubules with only moderate amounts of alkaline phosphatase. In this stage polarization of alkaline phosphatase activity in the brush border region is just beginning (fig. 6, 1). The mesonephros shows a strong and polarized alkaline phosphatase reaction in its secretory tubules.

Sections of 15-day embryos are characterized by the very intense alkaline phosphatase activity of the secretory tubules of the mesonephros and by the increase of this activity and its polarization in the secretory tubules of the metanephros (fig. 7, A). Alkaline phosphatase distribution in meso- and metanephros in the 17-day embryo presents no visible difference from the one just described.

A distinct change is apparent, however, in the mesonephros of 20-day embryos, where a clumping of the secretory tubule cells can be seen, as well as the shedding of alkaline phosphatase active particles (possibly cellular parts) into the tubular lumen (fig. 7, B). In the metanephros of embryos of this age a decrease in the activity of alkaline phosphatase can be observed in the brush border region of the secretory tubules (fig. 7, C). This diminution is very clear if one compares these slides with those of a 15-day embryo or, better, with those of a chick 6 days after hatching (fig. 7, D). Here the apical pole is very intensely stained along a relatively large band, indicating a greater alkaline phosphatase activity.

The study of slides stained with toluidin blue, with and without previous treatment with ribonuclease, showed in 5-day embryos a concentration of ribonuclease-removable basiphilic substance (R.R.B.) in the mesonephros. The tubules stained intensely although not to the same extent (fig. 7, E). There is no localized and constant intracellular distribution of R.R.B. The glomeruli always stained less than the tubules. Other organs which stained deeply at this age were the spinal cord, spinal ganglia, and liver.

In an 8-day embryo the mesonephric tubules stain with a much lighter colour, indicating a marked diminution of R.R.B. at this age. The Müllerian duct, however, presents a band of R.R.B. in the basal part of its cells (fig. 7, F). From this age onwards the R.R.B. of the mesonephros declines and becomes very scarce.

Sections of an 11-day embryo show the metanephric blastema and newly formed tubules staining darkly when compared with the adjacent mesonephros (fig. 7, G). In 15-day embryos the whole metanephros stains more darkly than the mesonephros, but several localized points situated in the periphery of the metanephros may be seen that stain still more intensely. These regions correspond to points where differentiation is still going on, as one can ascertain in haematoxylin-eosin stained slides.

Discussion

Wilmer (1943) demonstrated in normal and hydronephrotic kidneys a relation between phosphatase activity and kidney function. Reports in the literature as to the function of phosphatases in the kidney have been con-

flicting and were reviewed by Moog (1946), who stated on the basis of the evidence available: 'The conclusion that phosphatase is integrally concerned in renal function is thus inescapable, and the hypothesis that the enzyme is part of a glucose reabsorbing cycle is in good agreement with the facts.' This contention has been recently confirmed by March and Drabkin (1947), who demonstrated a correlation between blood sugar concentration and alkaline and acid phosphatase activity in the kidney. Furthermore, they obtained inhibition of both these enzymes by phloridzin *in vivo* and *in vitro*.

The possibility that the phosphatase activity of kidneys is correlated with the organ's function seems, therefore, very probable. The following discussion is based on this assumption.

Our data suggest that the beginning of *mesonephric activity* is between the 4th and 5th day of incubation, for at that period there is a concentration of alkaline phosphatase activity in the brush border zone of the secretory tubules. These findings concerning the beginning of secretion agree with Bakounine (1895), Lillie (1908), Atwell and Hanan (1926), Hanan (1927), Boyden (1927), Hurd (1928), Patten (1929), Chambers and Kempton (1933), Schneider (1939), and Moog's (1944) views on this subject. For a summary of their results see Table 2. It seems probable from our data that the mesonephros attains the peak of its activity between the 14th and 16th days. From then on the mesonephros not only loses weight, but its alkaline and acid-phosphatase activity are diminished. At hatching-time, however, there is still an appreciable amount of alkaline and acid phosphatase activity left. Our histochemical results for alkaline phosphatase agree with those of Moog (1944). These results do not confirm those of Lillie (1908), Hurd (1928), or Patten (1929), who place the beginning of the diminution of mesonephric activity between the 9th and 12th days of incubation. They are, however, in agreement with Fredericia (1912), Atwell and Hanan (1926), and Chambers and Kempton (1933), who state that the mesonephros activity declines only after the 16th to 18th days of incubation. Gersh (1937) observed that the mesonephros eliminates phenol red and ferricyanide up to at least the 14th day. He stated further that the decline of mesonephric activity, far from being abrupt, is a slow process. Our observations seem to corroborate this view.

As to the time of the onset of metanephros activity, our data are in accordance with those of a number of authors who contend that secretion begins after the 11th day of incubation (for a summary, see Table 2). At this date secretory tubules with alkaline phosphatase activity are present and our biochemical observations show a relatively rapid rise of alkaline and acid phosphatase activity at the same stage.

It is interesting to note that the peak of activity of both phosphatases is at about the 16th day followed by a decline from then on to the hatching time (figs. 4 and 5). A study of the literature showed that during this period the capacity of the metanephros to concentrate dyes is frequently lower than that of the mesonephros. These facts coincide with the reduction of the relative total kidney weight observed after the 14th day (fig. 3) and might suggest a

diminution of metanephric activity during the last quarter of the incubation period.

TABLE 2

Data on the Time of Beginning and End of Meso- and Metanephros Activity Collected from Literature

		Author	*Method used*	*Date*
MESONEPHROS	BEGINNING	Bakounine, 1895	Indigo carmine concentration	5th
		Lillie, 1908	Histological aspect	5th
		Atwell and Hanan, 1926	Trypan blue concentration	5th
		Hurd, 1928	Idem	5th
		Schneider, 1939	Idem	5th
		Chambers and Kempton, 1933	Phenol red concentration *in vitro*	4th
		Boyden, 1927	Experimental urinary stasis	4th
		Patten, 1929	Beginning of glomerulus circulation	5th
		Fisk and Boyden, 1926	Uric acid accumulation in allantoic fluid	5th
		Moog, 1944	Alkaline phosphatase distribution	4th
	END	Felix, 1906	—	9th–17th
		Patten, 1929	—	11th
		Lillie, 1908	—	10th–11th
		Fredericia, 1912	Size measurements	16th
		Schmalkhausen, 1926	Weight curve	14th
		Hurd, 1928	Idem	16th
		Atwell and Hanan, 1926	Diminution of trypan blue concentration	18th–19th
		Chambers and Kempton, 1933	Phenol red concentration	20th
METANEPHROS	BEGINNING	Lillie, 1908	Histological aspect	11th
		Hurd, 1928	Trypan blue concentration	11th
		Wislocki, 1921	Idem	13th
		Sandstrom, 1935	Idem	13th
		Gersh, 1937	Phenol red concentration	11th

The only known metabolic change in the chick embryo that could be correlated with these facts is the marked diminution of protein metabolism observed by Fredericia (1912) after the 16th to 17th day, Fisk and Boyden (1926) after the 14th day, and Needham (1926) after the 11th day.

The rapid increase of acid and alkaline phosphatase activity after hatching and consequent feeding of the animal suggests that there might be a correlation between food ingestion and phosphatase content in the metanephros. It seems to us that it would be of interest to feed or inject several foodstuffs of different nature in recently hatched chicks and study their action on the alkaline and acid phosphatase activity of the metanephros.

It is conceivable that the phosphatases play a role not only in the functional activity of the kidneys but also in their differentiation.

The presence of a strong alkaline phosphatase activity, diffusely located in the cells of the meso- and metanephric blastema and newly formed tubules, suggests such a correlation. Other examples of this fact were presented by Moog (1944).

The results obtained with Brachet's ribonuclease technique show a greater amount of ribonuclease-removable basiphilia in the early mesonephros (before the 6th day of incubation) when compared with the same organ later on. This finding suggests a correlation between the mesonephros differentiation and its R.R.B. content. We could thus strengthen Caspersson and Thorell's (1941) conclusions obtained with the ultra-violet absorption method. The metanephros presents, during its differentiation, concentration of R.R.B. in the blastema and newly formed tubules. This is particularly evident during the period between the 7th and 12th days of incubation and decreases from then on with the exception of certain groups of cells still in differentiation. These groups of cells persist up to the 20th day of incubation. Owing to this fact we are led to believe that differentiation is apparently more rapid and uniform in the mesonephros than the metanephros.

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Further Observations upon the Oxyntic Cells with Special Reference to Acid Phosphatase

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With two plates (figs. 2 and 3)

Summary

1. In continuation of work already reported (Menzies, 1949) further data are presented on the structure and cyto-chemistry of the granules in the oxyntic cells of the rat's stomach.

2. After multiple doses of pilocarpine or histamine, and after feeding, the phospholipine (as shown by acid haematein) is shed from some or all of the granules. The lipine first leaves the granules in cells situated in the basal part of the tubules, and finally those in the neck of the tubules, but a non-lipine lipoid remains in all the granules (as shown by sudan black).

3. The granules enlarge after prolonged starvation as they do after a single injection of pilocarpine; but after extraction of lipoids by hot pyridine and subsequent straining with iron haematoxylin, enlargement (i.e. of the non-lipoid moiety) is shown only after prolonged starvation.

4. A light, uncoloured central area is found in some of the largest granules after feeding and colouring with acid haematein.

5. An acid phosphatase appears in the oxyntic cells whose granules are about to lose their lipine component, and it disappears when they have done so. It is suggested that the acid phosphatase may cause hydrolysis of the phospholipine.

6. Only after prolonged starvation is there any apparent change in granule numbers, when they are decreased.

Introduction

IT has already been shown (Menzies, 1952) that the granules of the oxyntic cells in the rat's stomach contain a phospholipine and a non-lipoid component and, that after a single dose of pilocarpine or histamine the phospholipine moiety is shed from some of the granules in cells situated at the basal ends of the gastric glands. This observation suggested that loss of phospholipine from the granules was in some way related to the formation or excretion of HCl by the oxyntic cells, for pilocarpine, at least, is known to cause acid secretion in the rat. It was therefore decided to investigate further the action of pilocarpine and histamine on the granules by giving multiple doses of these drugs. The conditions of the granules after gastric secretion had been altered by prolonged starvation, and by feeding after a short period of starvation, was also investigated. Particular attention was paid to the phospholipine component of the granules (visualized by Baker's acid haematein method), and to the discovery of acid phosphatase activity in the oxyntic cells.

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It is the purpose of this paper, first, to show to what extent experimental procedures cause the phospholipine moiety to be shed from the granules, and to see if it can be shown once it has left the granules; and secondly to establish the presence of an enzyme, an acid phosphatase, within the oxyntic cells and to discuss its possible significance.

Materials and Methods

Male Norwegian rats averaging 200 mg. body weight were used. They were fed with oatmeal, dried milk, and cabbage before the experiments.

At the beginning of the experiments the rats were placed in separate cages and starved for 48 hours. Drinking water was freely available. Some were then killed as controls.

Twenty-one rats were starved for periods of 1–7 days, care being taken to prevent them eating their fur or faeces, or drinking urine which might have promoted gastric secretion. They were allowed water to drink and killed in batches of three at twenty-four hourly intervals. Sixteen rats were fed and killed in batches of four, after either 30, 60, 90, or 120 minutes from the beginning of the meal. Thirty rats were given intraperitoneal injections of pilocarpine nitrate (25 mg./kilo in 1 c.c. distilled water) at half-hourly intervals, and were killed in batches of three half an hour after their second to eleventh injection. Thirty rats were given intra-peritoneal injections of histamine hydrochloride (5 mg./kilo in 1 c.c. distilled water) at 45-minute intervals. They were killed in batches of three, 45 minutes after their second to eleventh injection.

Histochemical and cytological techniques

These were similar to those used in a previous paper (Menzies, 1952) and will only be listed here. Small pieces of mucous membrane from the fundus of the stomach were taken in all cases.

Tests for lipoids in general. (1) Sudan black (Baker, 1944, 1948), (2) Sudan II, (3) Sudan III, (4) Sudan IV.

Test for phospholipines. The acid haematein test (Baker, 1946). The non-lipoid moiety of the granules was stained with Heidenhain's iron haematoxylin after extraction of the lipoids with hot pyridine.

Tests for enzymes. (1) Lipase (Gomori, 1946), (2) Alkaline phosphatase (Gomori, 1939), (3) Acid phosphatase (Gomori, 1941).

Results

Control rats

In the present series of experiments the control animals were starved for 48 hours. In the previous experiments (Menzies, 1949, 1952), the controls were starved for 24 hours. The oxyntic granules after 48 hours' starvation (figs. 1, A; 1, B; 2, A; and 2, C) were in general slightly smaller than after 24 hours, but apart from this there were no appreciable differences. Negative

results were obtained with sudan II, III, and IV, and with tests for lipase. Alkaline phosphatase was present only in the nuclei of oxyntic cells. These results were similar in all the experimental animals and will not be referred to again. There was no trace of acid phosphatase present in control animals. The structure, distribution, and histochemistry of the granules in 'normal' rats (i.e. starved for 24 hours) has already been fully reported (Menzies, 1949, 1952).

FIG. 1. A, C, and E: typical oxyntic cells, whose granules are coloured with acid haematein. A, from control rat. The cell contains numerous spherical granules, of large, small and intermediate size. They are dispersed throughout the cytoplasm except in the intracellular canals shown near the nucleus; C, two hours after a meal. Except for an occasional large granule, the largest granules are smaller than the largest in the controls. A few granules with uncoloured central areas are seen, and a few are ovoid in shape; E, after 7 days' starvation. The largest granules have about twice the diameter of those in the controls. There are fewer granules present, and there is much less variation in their relative size.

B, D, and F: from camera lucida drawings of cells stained by iron haematoxylin after the extraction of lipoids. *Only a few of the largest granules are shown.* B, from control rat; D, two hours after a meal. This is indistinguishable from the control; F, after seven days' starvation. The non-lipoid moiety of the granules, as represented, is much larger than in the controls. G, an oxyntic cell (coloured by acid haematein) from a rat after its eighth injection of pilocarpine. A few granules still colour, the majority have lost their lipine component, and a large pool of lipine lies in the cytoplasm and possibly in the intracellular canals.

Fed rats

The granules were indistinguishable from those of the controls, $\frac{1}{2}$, 1, and $1\frac{1}{2}$ hours after the beginning of the meal, but after 2 hours changes were observed. With acid haematein the granules coloured less intensely than in the controls, although other structures (tissue eosinophils) in the sections coloured just as intensely. As in the controls, granules in some oxyntic cells at the basal ends of the gastric glands did not colour. There were more of these cells whose granules did not colour than in the controls, and this may indicate that a further release of phospholipine from the granules begins in this zone after two hours of gastric secretion. All the granules in all the cells,

however, coloured equally heavily with sudan black in both the fed animals and the controls, so that it would appear that non-lipine-lipoids are not affected by gastric secretion in the rat. Besides the loss of lipine, acid haematein showed that the majority of the larger granules (fig. 1, C) were smaller than those in the control animals. Very rarely a few larger granules were seen and some of these had uncoloured central areas like those seen after a single injection of pilocarpine. A few of the granules were ovoid also, whereas in the controls they were always spherical.

As in the controls, acid phosphatase was not demonstrated in any of the rats after feeding.

With iron haematoxylin (fig. 1, D), after the lipoids had been extracted with hot pyridine, the granules were indistinguishable from the controls. This is interesting when compared with the result as shown with acid haematein.

Starved rats

To describe changes in cell size after the daily periods of starvation an average was taken of the longest diameters of one hundred of the largest cells seen in each group. After 24 hours' starvation the oxyntic cells did not differ in size from the controls (18·8 μ), but after 72 and 96 hours they were markedly smaller (12·9 μ, 13·2 μ). After 120–168 hours' starvation (5–7 days) they returned to approximately the same size as the controls.

With acid haematein the largest granules appeared smaller after 48 hours (in all six animals) than after 24 hours (3 animals); they then increased in size in all animals up to 4 days, being larger on the third than on the first. Three animals were killed on the third and three on the fourth day. Of the rats killed, on the fifth and sixth days of starvation, four showed granules comparable to those killed on the fourth day. The other two were indistinguishable from the controls (the discrepancies may be explained by the animals having eaten their own hair or faeces, and thus stimulated gastric secretion). The largest of all granules occurred in all three animals after 7 days (figs. 1, E and 2, B), where they had about twice the diameter of those seen in the controls. The above sequence of change in granule size appeared also in the smallest granules and those of intermediate size from 1–6 days inclusive.

On the seventh days, however, in all rats the granules were more uniform in size than in any other rats so far observed. Only a few smaller granules were seen and none so small as in the controls. They were all discrete, and

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FIG. 2 (plate)

A. Two oxyntic cells (with nuclei) coloured by acid haematein to show the size of the largest granules in control rats. All the granules are spherical. Two peptic cells with granules are shown in the top right-hand side of the photomicrograph.

B. Acid haematein preparation of oxyntic cells from rat starved for seven days. No peptic cells are shown. Compare size of largest granules with those in A.

C. Iron haematoxylin preparation of oxyntic cells, after extraction of lipoids. Control rat.

D. Similar to C, but from rat after seven days' starvation. Compare granule size (non-lipoid component) with C.

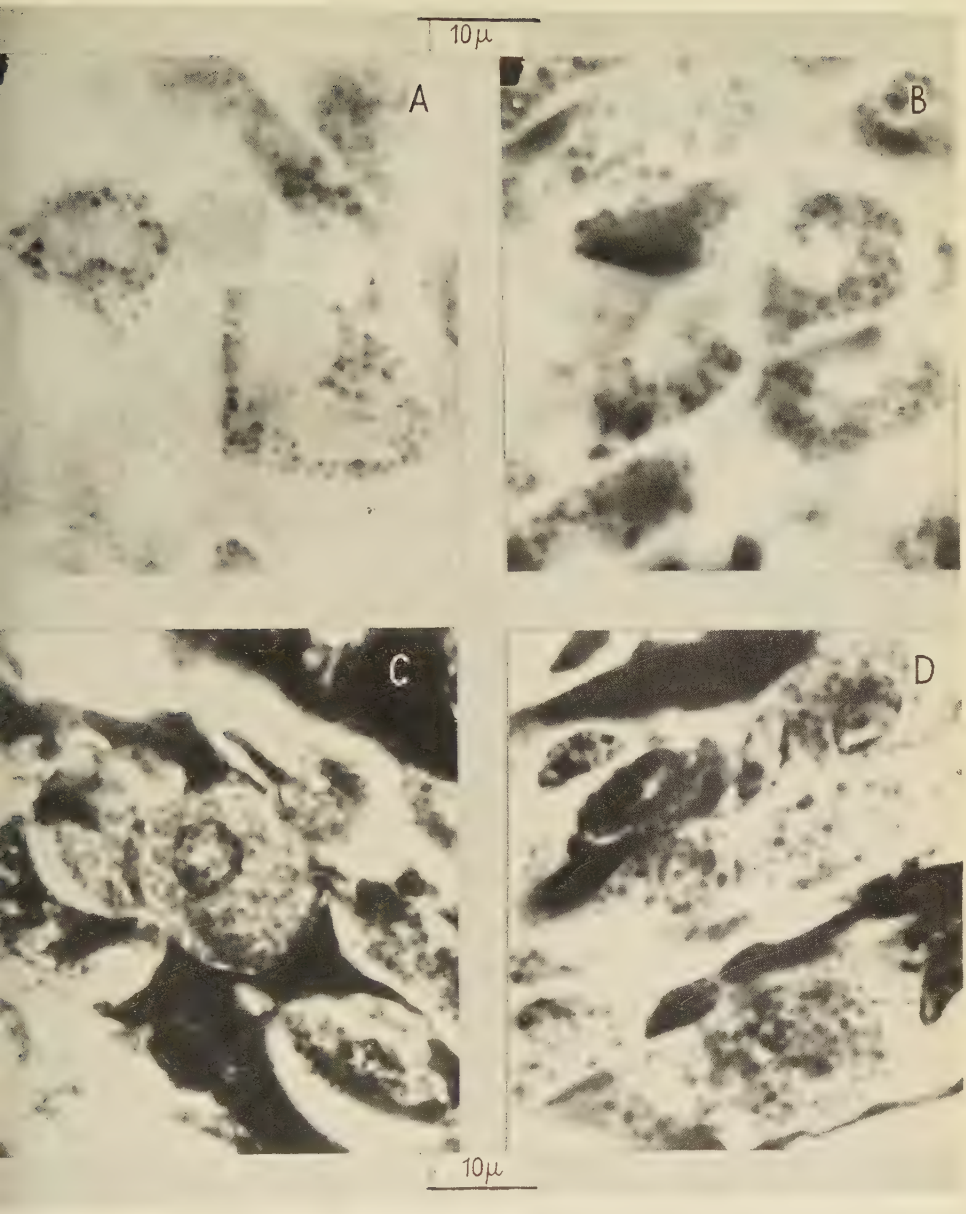

FIG. 2

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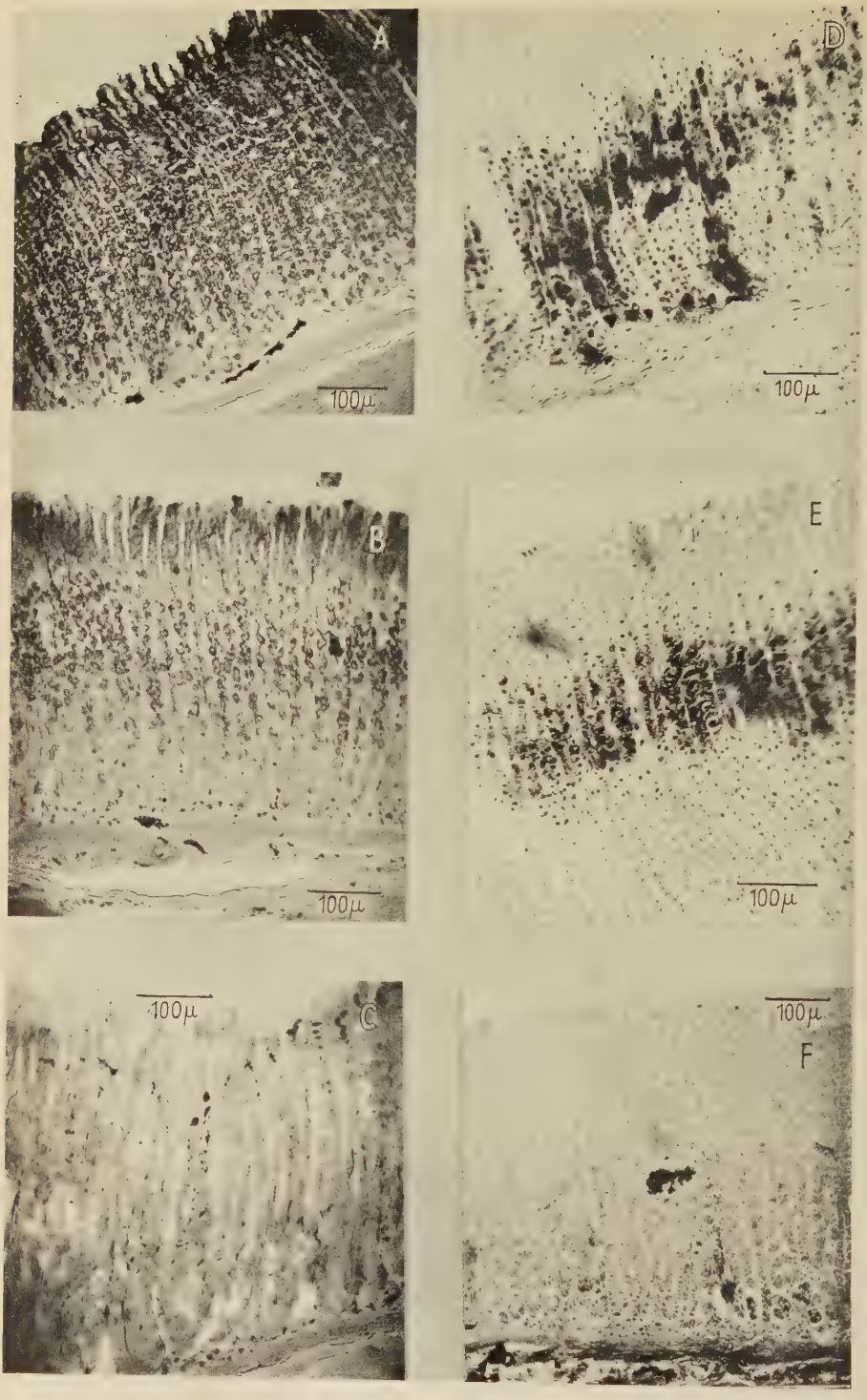

FIG. 3

GORDON MENZIES

spherical, and no uncoloured central areas were seen, as following pilocarpine and in those rats killed 2 hours after a meal. But, with regard to this, the large majority of the granules coloured very intensely, and undoubtedly some of the central areas were less densely coloured. Clumping of granules was rare on the seventh day, and they showed no peripheralization. Finally, in regard to granule numbers, they were probably equally numerous from 1 to 6 days inclusive, and less numerous after 7 days' starvation.

After extraction of the lipoids with hot pyridine, the non-lipoid moiety of the granules shown by iron haematoxylin became larger from the third to seventh days of starvation in those animals that showed granule enlargement with acid haematein, and on the seventh day (figs. 1, F and 2, D) the granule diameter was approximately twice the size of the largest seen in the controls. This result is interesting, as prolonged starvation is the only condition so far studied which causes an increase in the non-lipoid component of the granules.

The test for acid phosphatase failed to give any positive results with the starved animals.

Multiple injections of pilocarpine and histamine

The results with pilocarpine and histamine were very similar, so they will be described under the same heading. The general picture was of a progressive loss of phospholipine from the granules as shown by a failure to colour with acid haematein, and of the appearance of a layer of acid phosphatase active cells within the mucosa, whose enzyme retreats towards the lumen, *pari passu*, with the loss of phospholipine from the granules.

After the second injection of pilocarpine the granules in most of the cells at the bases of the gastric glands either coloured less intensely (with acid haematein) than the rest, or in many cases failed to colour at all. No acid phosphatase was demonstrable. After three or four injections, acid haematein showed (fig. 3, B—compare with fig. 3, A) a zone of uncoloured granules which

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FIG. 3 (plate)

A, B, C. Acid haematein preparations of gastric mucosa.

D, E, F. Sections stained for acid phosphatase and counter stained with safranin.

A. Control rat. To show the extent to which the granules colour with acid haematein. Only a few oxyntic cells, deep in the bases of the glands near the musculavis mucosae, have granules which do not colour.

B. After three injections of pilocarpine. The granules in most of the oxyntic cells in the basal half of the mucosa have shed their lipine, and also in many cells extending upwards towards the neck region of the gastric glands.

C. After ten injections of pilocarpine. Almost all the oxyntic granules throughout the mucosa have shed their lipine and fail to colour. Other structures that colour with acid haematein (red blood-cells and tissue eosinophils) do so as intensely as they do in A and B.

D. After four injections of pilocarpine showing acid phosphatase present in a band of cells (the great majority of which are oxyntic cells) in the neck region of the gastric tubules, and in oxyntic cells extending down to the bases of the glands in many of the tubules.

E. Following six injections of pilocarpine, acid phosphatase is present in a band of oxyntic cells limited to the neck region of the gastric tubules.

F. After eight injections of pilocarpine. Acid phosphatase has vanished from all the oxyntic cells in the whole section save for the one area shown in the middle of the photograph.

spread from the base of the glands towards the neck, where the remaining coloured granules were somewhat smaller than the controls and were more uniform in size. Throughout this zone the granules in the oxyntic cells in some glands still coloured whilst those in adjacent glands did not. Under low power this led to the appearance of a band of darkly coloured oxyntic cells in the neck region of the glands, with here and there prolongations downwards of similar darkly coloured cells. Also, after three or four injections of pilocarpine, acid phosphatase appeared in a band in the neck region of the gastric glands with streaks of activity spreading here and there in oxyntic cells towards the basal region (fig. 3, D). In the band just described the acid phosphatase activity, under high power, was seen chiefly to be concentrated in the cytoplasm, nuclei, and granules of the oxyntic cells. Apart from this, it was seen in many other nuclei and many adjacent connective tissue fibres and possibly in some adjacent mucoid cells. Occasionally, in some sections, it was seen in some muscle cytoplasm, in muscle fibre nuclei, and in the endothelium of small arteries. There was a general correspondence between the position of the cells exhibiting this activity and that of cells showing greatest coloration with acid haematein.

After five or six injections none of the granules in the basal half of the tubules coloured with acid haematein, and this left a band of positively reacting 'cells' in the neck region, extending from end to end of the sections. A band of acid phosphatase positive cells, the vast majority of which were oxyntic cells, was present in the neck region of the gastric tubules (fig. 3, E). There was no acid phosphatase present in the basal half of the glands.

After seven to nine injections the above band as shown by acid haematein became narrower and in many places ceased to exist where all the granules in all the cells in many glands failed to colour. A similar picture (fig. 3, F) was shown with the test for acid phosphatase. Also, what appeared to be a release of phospholipine into the general cytoplasm, or its accumulation in intracellular canals was observed, for blue-black pools were demonstrated by acid haematein (fig. 1, G) in all of the animals save one in this group, a condition never observed in the controls, or in other rats so far investigated.

After ten to eleven injections, the oxyntic granules throughout the mucosa failed to colour with acid haematein (fig. 3, C) save for an occasional cell here and there in two of the rats. The cells still contained numerous uncoloured granules seen by virtue of their refractility. Red blood-cells, and other cells that colour with acid haematein, did so to the same intensity as in the controls. In this group of animals acid phosphatase activity was either completely absent or demonstrated in an occasional oxyntic cell in the neck of the glands.

Discussion

It seems from the above results that the phospholipine of the oxyntic granules is stable in control animals and during prolonged starvation, but that gastric secretion whether induced by feeding or pilocarpine results in a loss of phospholipine from the granules. This starts in the oxyntic cells at the

basal ends of the gastric glands and spreads towards the neck region, until finally no phospholipine remains. A similar, but somewhat less marked picture, was seen after many injections of histamine, although it is doubtful if this drug produces acid secretion in the rat. In cells whose granules are in the process of losing their phospholipine, acid haematein occasionally shows that phospholipine is lying diffusely within the cell. So far as I am aware, these results with acid haematein constitute the first definite evidence that lipine is split off from the granules, but this has previously been suggested (though not adequately proved) by Ma *et al.* (1927), using histamine. These writers say that 'The mitochondria [by which they mean the oxyntic granules] dissociate separating the lipoid component from the non-lipoid during the act of secretion', asserting that the former is responsible for basiphilic material which accumulates in the region of the intracellular canals. Since, however, their preparations claiming to show basiphilia were stained with Mallory's triple stain, which consists of three acid dyes, little weight can be attached to their statement on this particular point.

The question arises as to how the granules lose their lipine. Gomori, using his histochemical test for acid phosphatase, stated that it was negative so far as the gastric mucosa was concerned. This statement is not wholly true, for the above experiments show that after a certain number of injections of pilocarpine or histamine, an acid phosphatase appears in the nuclei and granules of oxyntic cells. It seems from these experiments that the acid phosphatase makes its appearance just before the lipine is about to be removed and is present whilst it is being removed (and possibly for some short time after it has all gone), for in any one section it is only demonstrated in a few of those cells situated in the bases of the tubules, where the lipine is removed very rapidly, but it is present over a period of hours in those cells superficially situated where the lipine disappears more slowly. It seems, therefore, that an acid phosphatase may cause the hydrolysis of the phospholipine, and further support to this suggestion, based on work at present in progress, will be published later. If this enzyme brings about hydrolysis of the phospholipine, it would presumably produce phosphoric acid, a nitrogenous base, and a diglyceride, and as none of these substances are shown by acid haematein the question arises why, in some cases, a diffuse black substance is demonstrated by acid haematein lying within the cell. A non-lipoid part of the granule exists (this will be discussed later), and contains protein (Menzies, 1952). I would suggest that the phospholipine part is linked either by its phosphoric acid radicle, or by its nitrogenous base to the protein part, by an ionizable bond, and that the phosphoric acid produced by enzyme action causes a change in pH which breaks this bond, so that not only does one get the products of hydrolysis of the phospholipines in the cytoplasm, but also free phospholipine—and this is the black substances demonstrated by acid haematein. The above suggestion that the acid phosphatase acts as a hydrolyser of the phospholipines seems to me to be the more probable role of the enzyme in the oxyntic cell. Nevertheless, one must bear in mind the fact that

although an extracted phosphatase will hydrolyse phospholipines in a test-tube it is possible that in the living cell it is not concerned in hydrolysis of phosphate esters, but that it provides focal points for the release of energy required for protein contraction and extension as suggested by Danielli (1951), when reviewing certain theories of Goldacre and Larch (1950).

It would now be well to consider what is left of the granules after the lipine has disappeared, for they are still visible by virtue of their refractility, and stain with Heidenhain's haematoxylin. In all cases sudan black shows a lipoid to be present; thus the granules contain a non-lipine lipoid, a fact previously suggested (Menzies, 1952). After complete extraction of the lipoids by hot pyridine, iron haematoxylin demonstrates that the non-lipoid part still exists, and is indistinguishable from that in the controls.

The size and numbers of the granules now remain to be considered. After prolonged starvation (as after a single dose of pilocarpine or histamine—Menzies, 1952), the granules increase in size. The question at once arises whether the increase in size is due to enlargement of the lipoid part, the non-lipoid part, or both. After a single dose of pilocarpine or histamine it would appear to be the lipoid moiety alone that changes in size; for the residue after staining with iron haematoxylin appears to be similar in all respects to the controls. A different picture is present, however, after prolonged starvation. Here acid haematein shows that the granules enlarge, but at the same time the residue has increased in diameter to approximately twice that of the controls. These results afford an interesting contrast although little can be said at the moment about their significance. It certainly seems as if there are substances—pilocarpine and histamine—which produce an increase in the amount of the lipoid component without affecting the non-lipoid part, whereas prolonged starvation appears to bring about an increment in the non-lipoid part. On the assumption that the non-lipoid part is principally protein, its enlargement might be due either to a synthesis of more protein, or to a hydration of that already present. In connexion with the former alternative it can be pointed out that with methyl green and pyronin there was no demonstrable cytoplasmic basiphilia in the cells of starved animals such as might be associated with pentose-nucleic acid which, according to Brachet (1947), Caspersson (1947), and others, are the common concomitants of active protein synthesis.

Finally, with regard to granule numbers, Gitlitz and Levison (1936), using rabbits, report a marked decrease after pilocarpine. This does not occur in the rat after single or multiple doses of pilocarpine. Babkin (1944) states that the granules do not decrease in number during secretion and, except after prolonged starvation, the results of my experiments are in accord with this view. However, there almost certainly is a decrease in the number of granules present after prolonged starvation (where the cells are the same size as in the controls), but so great is the number of the granules present and so closely are they packed together that no counts have been possible. There is no evidence to suggest how the granules decrease in number, whether by

coalescence, or by expulsion from the cell. They have not been seen in the process of coalescing, nor has iron haematoxylin shown the non-lipoid part to be present outside the cell—in the tubules for examples.

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Interstitial Cells in the Regeneration of *Cordylophora lacustris*

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With two plates (figs. 6 and 7)

SUMMARY

1. Histological examination of developing reconstitution masses of *Cordylophora acustris* has shown that interstitial cells accumulate in the tips of developing outgrowths, and that in contrast to other regions these interstitial cells are situated in he endoderm.

2. These interstitial cells do not differentiate into the ectoderm and endoderm of he regenerant, but persist and increase in the adult oral cone.

3. Interstitial cells do not accumulate in regions of growth (stolon tips) or growth nd differentiation (induced outgrowths) that do not contain the rudiment of an oral one.

4. An invariable association has been established between the presence of an interstitial cell accumulation in the endoderm and hydranth-inducing power of tissue when grafted to a mass. Outgrowth tips and oral cones both have inducing power and nterstitial cell accumulations; basal hydranth regions and tentacles have inducing power, and although there is no interstitial cell accumulation in these regions of the ntact hydranth, it appears before a graft produces induction.

5. There is evidence that interstitial cells are particularly rich in ribonucleic acid. Whether this property is directly related to the function of induction has yet to be determined.

AN experimental investigation of the induction of regeneration in the hydroid *Cordylophora lacustris* has been described in an earlier paper (Moore, 1952). The results of the experimental work may be summarized as follows. First, *Cordylophora* reconstitution masses, made by chopping up unspecialized tissue and piling the fragments into heaps, may regenerate hydranths 'spontaneously'. An oral cone grafted into a mass induces the development from the tissue of the mass of hydranth regions basal to the oral cone. Regeneration of the mass at points other than the graft site is inhibited.

Second, the inducing properties of oral cone grafts are shared by grafts of other regions of the hydranth (tentacular ring, subtentacular region, hydranth neck and even small fragments of tentacle), and by the rudiment of the developing hydranth (tips of outgrowths from masses), but not by tissue which does not possess hydranth differentiation (stem coenosarc and stolon

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tip grafts). The regions of the hydranth are defined in figures in the earlier paper.

Third, no diffusing chemical inductor has been detected by using agar or cigarette paper barriers between the graft and host, and there is evidence that direct close contact between the graft and host tissue is needed for induction to take place. No inductions have been obtained with killed or macerated grafts, but fragments or disorientated pieces of differentiated tissue may produce induction.

It may be concluded that hydranth induction is a property of differentiated tissue in direct close contact with unspecialized tissue.

The present paper gives an account of the histological examination of masses with and without grafts, fixed and sectioned at different stages of development. A correlation is revealed between an inducing ability of tissue and the presence of a particular formation of interstitial cells. The name 'mass body' will be given to the main part of a reconstitution mass, as opposed to outgrowths from or grafts upon it.

Earlier work on the histology of regeneration in hydroids

There would seem to be two possible sources of regenerated tissue: differentiated ectoderm and endoderm cells, or unspecialized interstitial cells. The cells may divide at the site of regeneration, or divide throughout the animal and migrate to the site of regeneration, and there differentiate into the various cell-types and become rearranged into the new organism.

Unspecialized cells are important in the regeneration of many animals (Stolte, 1936, general review; and Wolff and Dubois, 1948, for planarians). In hydroids, however, the importance of interstitial cells in budding and regeneration has been greatly disputed. Lang (1892), Tannreuther (1909), Hadzi (1910), Gelei (1925), and other early workers agreed that bud formation in hydra is initiated by accumulation and division of interstitial cells in the ectoderm at the bud site. In the closely allied process of regeneration, however, results with the same technique (mercuric-acetic fixation and haematoxylin staining) did not support the idea that interstitial cells are an important source of new tissue. Rowley (1902) observed mitoses in ectoderm cells throughout regenerating hydras, and Mattes (1925) and Kanajew (1926) established that regenerating hydras show a greater number of mitoses than unoperated animals. No local proliferation of interstitial cells was seen. Work on *Tubularia* also (Bickford, 1894; Stevens, 1901; Godlewski, 1904) had not emphasized the importance of interstitial cells in regeneration.

The controversy about the function of interstitial cells in regeneration turned largely upon whether migratory or mitotic activity of differentiated cells was detected. Workers continued to disagree about the occurrence and frequency of divisions in ectoderm and endoderm cells. McConnell (1932, 1933) described mitoses in all types of cells, and his illustrations suggest a source of disagreement: his 'dividing epithelial cell' is identical with the 'interstitial cell' of some other authors, notably Schlottke (1930). Differences

n nomenclature may account for the differences of opinion, but whether differentiated cells are among the cells seen dividing remains uncertain. That differentiated cells contribute to regeneration by migration is suggested by observation of regenerating tissue, and was established by vital dye marking (Goetsch, 1929; Kanajew, 1930; Hammerling, 1936).

The strongest support for the interstitial cell theory of regeneration is provided by irradiation experiments. Zawarsin (1929) exposed hydras to suitable doses of X-rays and found that head regeneration was then inhibited. Strelin (1929) followed up Zawarsin's work histologically and obtained a close correlation between the disappearance of regenerative ability and the destruction of interstitial cells. Further evidence that irradiated tissue cannot regenerate, although all but the interstitial cells are apparently unharmed, was provided by Puckett (1936) and Evlakhova (1946). Great caution is needed in drawing conclusions, however, since the effects of irradiation are not fully known. The work of Wolff and Dubois (1948) on destruction of unspecialized cells by irradiation in planarians does provide conclusive evidence for the importance of unspecialized cells in the regeneration of these animals.

Some of the more recent work on hydroids, however, suggests that the ectoderm and endoderm of the regenerant does not originate from differentiation of interstitial cells. Kanajew (1930) used Giemsa's eosin-azur stain, which distinguishes interstitial cells very clearly. He found that regions poor in interstitial cells, such as the stalk of *Pelmatohydra*, could regenerate foot but not head regions (supported by Tokin, 1934). Kanajew suggested that interstitial cells are necessary for the tentacles. There is no evidence to support this suggestion; on the contrary Persch (1933) reported similar phenomena in *Microhydra* which has no tentacles. Indirect evidence was provided by Beadle and Booth (1938) and Papenfuss and Bokenham (1939) who found that reconstitution masses made from either ectoderm or endoderm alone cannot regenerate: the interstitial cells in one layer cannot provide the differentiated cells of the other.

Methods

The results of the present investigation show that while interstitial cells play an important part in the regeneration of *Cordylophora* masses, their action is not, as the interstitial cell theory assumes, to differentiate into the ectoderm and endoderm of the regenerant.

Reconstitution masses of *Cordylophora* were fixed at successive stages of regeneration, cut into serial sections of standard thickness and stained with Giemsa's eosin-azur stain, which distinguishes interstitial cells particularly clearly (Kanajew, 1930). Counts were made of the number of interstitial cells per section and the area of the sections recorded, to obtain quantitative data on the density of interstitial cells in the different regions of regenerating masses.

Material was fixed in Zenker's fluid. After impregnation by Peterfi's

celloidin-paraffin method, paraffin of M.P. 52° C. was used for embedding, and serial sections were cut 8 μ thick. The following staining technique proved satisfactory:

Giemsa's eosin-azur stain, freshly diluted (1 drop of stock solution per c.c. of distilled water)	60 min.
Distilled water	20 sec.
0·5 per cent. acetic acid	20 sec.
Distilled water	20 sec.
70 per cent., 90 per cent., first and second, absolute alcohols	10 sec. each

Clear in xylene, mount in neutral balsam.

Interstitial cells can be clearly distinguished as small cells with dark blue cytoplasm, large colourless nucleus, and dark nucleolus. The number of these cells in each section has been counted under a one-sixth inch objective. Interstitial cells have an average diameter of 5 μ; therefore, in examining a section 8 μ thick counts were made without altering the focus. Interstitial cell fragments were recognized when the nucleus and some cytoplasm was present. Counting errors due to uncertain identification, enucleate fragments, and similar factors were considered to be unimportant in relation to the variability of cell numbers which was found.

There are some apparent mitoses in interstitial cells in masses at all stages of development, but mitoses are difficult to detect after Giemsa staining. No intense localized interstitial cell proliferation was seen.

The number of interstitial cells was counted in each section of most of the masses. To measure the area of sections, camera lucida drawings were made of about every fourth section. From these drawings the average radius of the whole section and the average width of the tissue was measured, and the area of tissue calculated by subtraction of the area of the coelenteron from the area of the whole section. In calculating the 'density', the number of interstitial cells per unit area of section, for convenience 1,000 sq. μ was taken as the unit of area.

In fig. 1 the interstitial cell distribution in the sections of four masses at the spherical-stage (before hydranth regeneration begins) is shown graphically, to illustrate the variation in interstitial cell distribution in uniform tissue. Since the number of interstitial cells is seen to vary $\pm$ 10 between adjacent similar sections, significant variations must be strikingly greater than this.

The interstitial cell distribution in masses at later stages of development is found to have certain well-defined characteristics (figs. 2–7): average values of interstitial cell density ($\triangle$) in a given region at a given stage are recorded. Since masses made from different pieces of tissue contain widely different numbers of interstitial cells, however, the interstitial cell densities characteristic of the different regions are more clearly seen by comparing interstitial cell densities within each mass separately.

The Interstitial cell distribution in developing Masses

Spherical stage. Masses reach the spherical stage of development after about 4 hours. The ectoderm and endoderm cells have been sorted out into their normal relative positions and the coelenteron has been formed, but there are no signs of outgrowths.

The interstitial cells are found mainly at the inner border of the ectoderm, and their distribution is irregular throughout the mass (fig. 1).

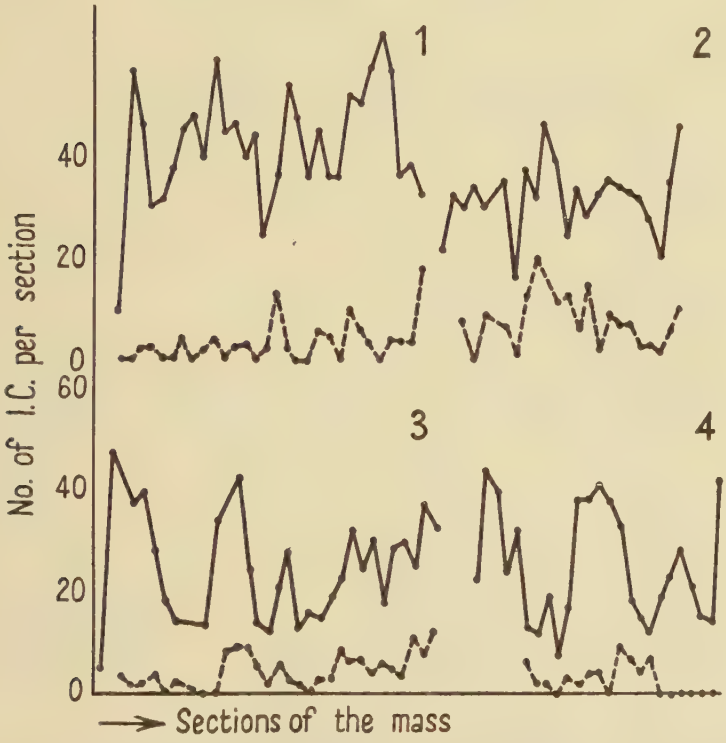

FIG. 1. The distribution of interstitial cells (I.C.) in four spherical stage masses. Continuous lines represent ectoderm, dotted lines endoderm.

Out of 11 spherical stage masses, each with 30 to 45 sections:

Extreme recorded values of △	1·8 and 16·6
Mean value of △	8·0

In all the sections more than half the interstitial cells are in the ectoderm.

Outgrowths. After the spherical stage, regenerating masses either produce 'outgrowths' which later differentiate into hydranths or produce stolons attached to the substratum which later bud off hydranths laterally. Fifty-eight outgrowths, of the type which directly differentiate into hydranths, have been examined histologically: the examination revealed an accumulation of interstitial cells at the outgrowth tips.

The interstitial cell distribution in a typical mass with two outgrowths is shown graphically in figs. 2 and 3. In this mass,

Range of △ in mass body		12–20
,, ,, at base of outgrowth	(1)	6–10
,, ,, ,, ,, ,,	(2)	3–8
,, ,, at tip of outgrowth	(1)	10
,, ,, ,, ,, ,,	(2)	39

(see fig. 3).

FIG. 2. The distribution of interstitial cells (I.C.) in a mass with two outgrowths.

The interstitial cell density is relatively low at the bases of the outgrowths. There is an intensive accumulation of interstitial cells at the tip of the second outgrowth (density 39), which is the most important aspect of the interstitial cell distribution in masses with outgrowths.

As at the spherical stage, in the mass body and at the bases of the outgrowths the interstitial cells are mainly in the ectoderm. In the second outgrowth tip

however the accumulation of interstitial cells is in the endoderm (see fig. 2). Although there is not a density peak in the tip of the first outgrowth, in this outgrowth tip also more than half of the interstitial cells are in the endoderm. Photographs of sections from the mass body, outgrowth base, and outgrowth tip are given in fig. 6, A, B, C, E.

FIG. 3. The interstitial cell (I.C.) density in a mass with two outgrowths.

Table 1 shows the proportion of the outgrowths examined which have peaks of interstitial cell density at the tip, and the proportion which have more than half of the interstitial cells located in the endoderm at the tip. These results show that an accumulation of interstitial cells in the endoderm is typical of developing outgrowth tips.

The absence of interstitial cell accumulations in the earliest outgrowth tips (Table 1, B) suggests that the accumulations are gradually acquired as the outgrowths develop. The greatest accumulations are found in the oldest and longest outgrowths.

Masses with fully formed hydranths or with development induced elsewhere

by a graft may also have outgrowths, but these outgrowths are inhibited from developing into hydranths. These outgrowths do not have interstitial cell accumulations at the tips (Table 1, C). Where masses have more than one outgrowth, the absence of an interstitial cell accumulation in one of these outgrowths may be the result of the inhibition of one outgrowth by the other. Eight of the 12 outgrowths tabulated in Table 1, A as lacking interstitial cell accumulations are from masses with more than one outgrowth. The first outgrowth from the mass illustrated in figs. 2 and 3 is included among these. All but three of these outgrowth tips (Table 1, A) have the first sign of an interstitial cell accumulation in that more than half of the interstitial cells are in the endoderm.

TABLE 1

Interstitial Cell (I.C.) Density (△) Peaks in the Endoderm (End.) of Outgrowth Tips

Total no. of outgrowths examined	*△ peak at tip*		*Location of I.C. majority at tip*	
	Present	*Absent*	*End.*	*Ect.*
A. Outgrowths apparently developing				
29	17	12	26	3
B. Outgrowths from masses only 24 hours old				
14	2	12	4	10
C. Outgrowths inhibited by inductions or by adult hydranths				
15	0	15	2	13

While different masses vary, the scale of the interstitial cell accumulations may be indicated by the following average figures:

Average △ in the mass body of the 29 masses with developing outgrowths (A) . . . . . 9·7
Extreme recorded values . . . . . 4 and 20
Average △ in the bases of 17 developing outgrowths (A) where a density peak is found . . . . . 7·6
Extreme recorded values . . . . . 2 and 15
Average △ in the tips of 17 developing outgrowths (A) where a density peak is found . . . . . 21·6
Extreme recorded values . . . . . 10 and 40

Histological examination shows that outgrowths developing into hydranths have interstitial cell accumulations in the endoderm at the tip, and that the density of the accumulations increases as the outgrowths develop. This result might be taken to support the hypothesis that regenerated material is derived from interstitial cells. After masses at the hydranth stage of development had been examined, however, the accumulation of interstitial cells at the tips of developing outgrowths was seen to bear quite a different interpretation.

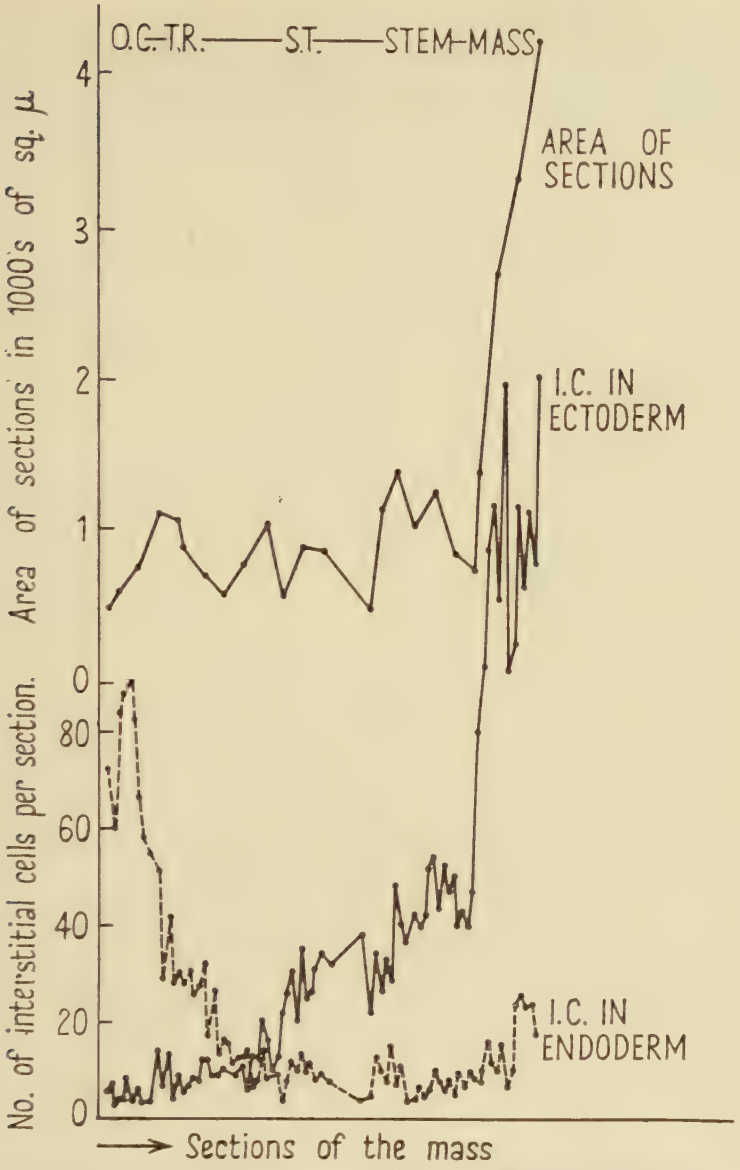

FIG. 4. The distribution of interstitial cells (I.C.) in a mass with a hydranth. O.C., oral cone; T.R., tentacular ring; S.T., subtentacular region.

Hydranths. The interstitial cell accumulation of the outgrowth tip was found to persist and increase in the oral cone of the regenerated hydranth, contrary to the assumptions of all previous workers. The interstitial cell distribution in a mass with one fully formed hydranth is shown graphically in figs. 4 and 5. The average value of ∠ in the different regions of ten masses with hydranths are given in Table 2, A.

The interstitial cell distribution in the mass body at this stage is similar to that of the mass body at the outgrowth stage, but with a higher proportion of interstitial cells. As the figures above show, there is no fall in interstitial cell density in a hydranth stem to correspond with the fall in density at the

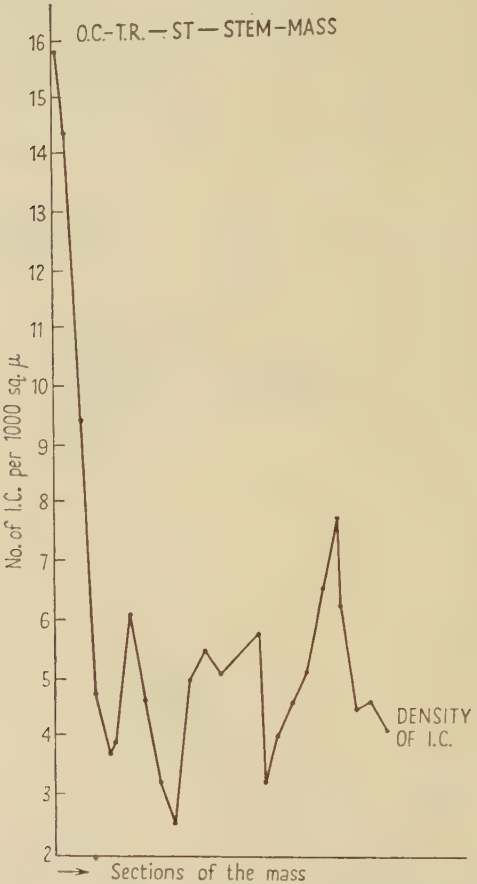

FIG. 5. The interstitial cell (I.C.) density in a mass with a hydranth. Abbreviations as in fig. 4.

base of an outgrowth. There is a region of lower interstitial cell density in the subtentacular region of the hydranth, where the outer part of the endoderm is entirely vacuolated, with occasional radially elongated interstitial cells between the vacuoles (fig. 7, c).

In the tentacles interstitial cells may be present or absent, but are usually present at the tentacle bases in the ectoderm. The area of cross-section of a tentacle is too small for comparison of densities with other tissues.

The concept governing the whole of this study was altered on finding a dense accumulation of interstitial cells in the endoderm of the adult oral cone.

While in the basal regions of a mass with a hydranth the interstitial cells are mostly in their normal ectodermal position, in the tentacular ring more than half the interstitial cells are in the endoderm, and in the oral cone the interstitial cells are usually confined to the endoderm. The interstitial cell accumulation in the oral cone is as great as, or greater than, that of the tip of a developing outgrowth.

TABLE 2

The Interstitial Cell Distribution in Masses with Hydranths and Induced Hydranths

Average Values of △ are given, and the Density Range Indicated

	Mass body	*Stem*	*Subtentacular region*	*Tentacular ring*	*Oral cone*
A. 10 spontaneously regenerated hydranths . .	15·2 (6–30)	19·4 (5–35)	9·5 (4–16)	13·3 (6–20)	36·7 (26–49)
B. 10 hydranths induced by oral cone grafts . .	12·3 (4–29)	7·7 (4–12)	6·4 (3–12)	10·5 (3–22)	30·1 (20–40)
C. 10 hydranths induced by grafts other than the oral cone	12·0 (4–26)	12·3 (2–27)	10·6 (2–12)	11·8 (2–24)	32·7 (21–58)

Photographs of transverse and longitudinal sections of oral cones are compared with photographs of sections of outgrowth tip in fig. 6, C and D, E and F. Unlike outgrowth tips, oral cones have a well-defined structure. The interstitial cells are not only accumulated in the endoderm but arranged in a characteristic form. The endoderm is separated into blocks, leaving a T-shaped or star-shaped coelenteron, and the interstitial cells are radially elongated and concentrated at the outer border of the endoderm. The ectoderm is characteristically free from interstitial cells.

To establish that this description is typical of oral cones, sixty oral cones of widely different ages have been examined. Of these, two were faultily stained. The remaining fifty-eight oral cones all had interstitial cell accumulations in the endoderm:

Out of fifty-eight oral cones:

Mean I.C. density	33·29
I.C. density range	16–56

Out of seventeen developing outgrowth tips (see above)

Mean I.C. density at tip	21·6
I.C. density range	10–40

These results show that the interstitial cells which accumulate in the tip of a developing outgrowth do not differentiate into the ectoderm and endoderm of the hydranth, but persist and increase in the adult oral cone. The function of these cells at the growing point is clearly not in this instance the

provision of new material for the regenerant, as has previously been assumed, but the formation of a part of the adult organization.

This conclusion was confirmed by histological examination of stolons and induced outgrowths and hydranths: regions of growth and differentiation which do not contain the oral cone rudiment were found not to have interstitial cell accumulations.

Stolons. A stolon does not become directly transformed into a hydranth at the tip but laterally buds off an outgrowth which develops into a hydranth. The stolon tip therefore is a growing point, but is not the site of a future oral cone. If interstitial cells are required as materials for growth, interstitial cell accumulations might be expected at the tip of a developing stolon. If interstitial cells accumulate to form the rudiment of the adult oral cone, an accumulation would not be expected in a stolon tip but only in the outgrowth budded off by the stolon.

Out of sixteen stolons:

Region	*I.C. density range*	*Mean I.C. density*
Mass body	4–33	14·2
Stolon base	5–45	13·2
Stolon tip	0–26	9·5

In none of the stolons is there an interstitial cell accumulation at the tip. In one stolon only, more than half the interstitial cells at the tip are in the endoderm. In the other fifteen stolon tips the interstitial cells are in their normal ectodermal position (fig. 7, A).

In the stolon tip the cells are characteristically vacuolated. Berrill (1949) associated vacuolation in the cells of *Obelia* with regions of active growth, and the same association may exist here.

The two stolon buds which were examined are entirely similar to directly developing mass outgrowths, with interstitial cell accumulations in the endoderm at the tip.

Induced outgrowths. An outgrowth induced by an oral cone graft is not only a region of growth, as is the stolon tip, but it is the region from which differentiate all the parts of the hydranth basal region to the oral cone. Unlike the tip of a 'spontaneous' outgrowth, it does not, however, contain the oral cone rudiment, because the oral cone of the adult hydranth is provided by the graft. If the function of the interstitial cells were to provide regenerant tissue, an

FIG. 6 (plate). The figure shows the aggregation of interstitial cells (darkly stained with Giemsa's stain) in the endoderm of the outgrowth tip and the oral cone.

A. T.S. mass body.
B. T.S. outgrowth base from the same mass.
C. T.S. outgrowth tip, ditto.
D. T.S. oral cone of adult hydranth.
E. L.S. outgrowth tip.
F. L.S. oral cone.

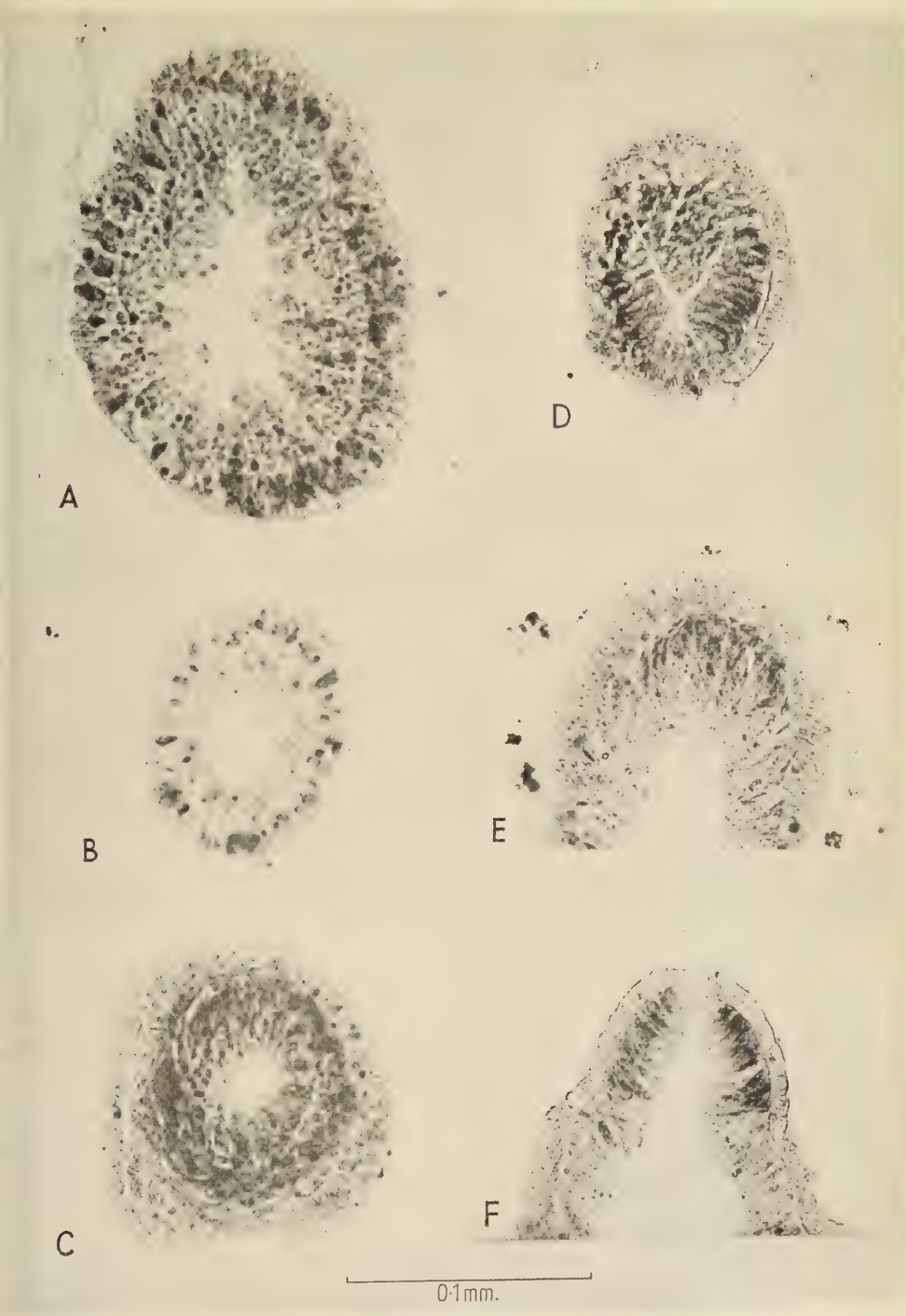

FIG. 6
J. MOORE

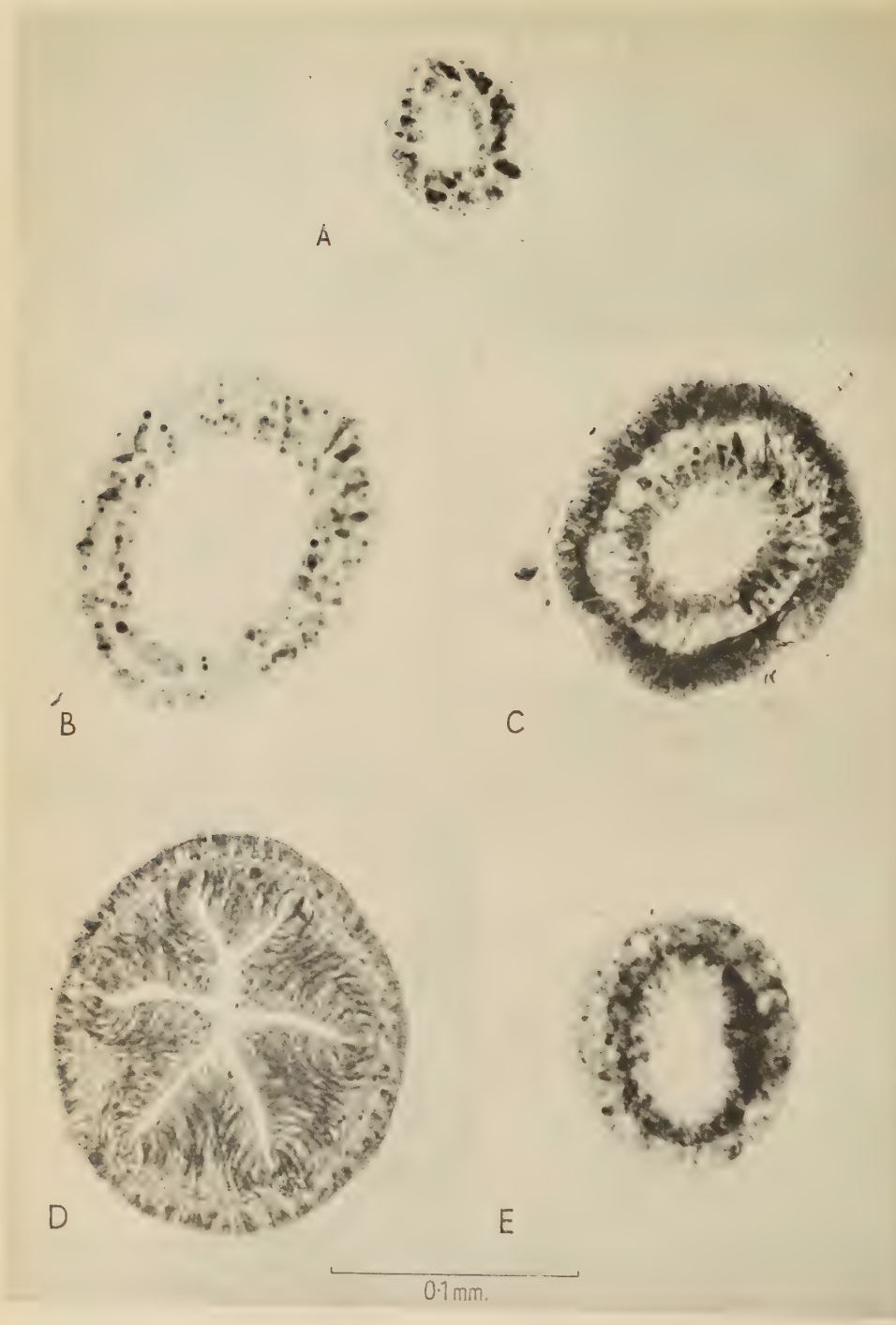

FIG. 7
I. MOORE

accumulation of interstitial cells would be expected in an induced outgrowth. If the function of the interstitial cells were to provide the rudiment of the oral cone of the hydranth, no accumulation would be needed in an induced outgrowth.

The interstitial cell distribution in an induced outgrowth is illustrated graphically in fig. 8. Out of twenty-one induced outgrowths:

Region	*I.C. density range*	*Mean I.C. density*
Mass body	2–24	8·5
Induced outgrowth	2–22	8·8
Oral cone graft	16–28	22·5

From these figures it will be seen that there is no interstitial cell density peak in an induced outgrowth. The density is similar to that of the mass body, and the interstitial cells are situated in the ectoderm (fig. 7, B). There is no trace of the ectodermal accumulation of interstitial cells found in 'spontaneous' outgrowths (fig. 6, C). Induced outgrowths are regions of growth and differentiation entirely comparable to spontaneous outgrowths, with the difference that the oral cone of the future hydranth is derived from the graft.

Induced hydranths. The interstitial cell distribution in induced hydranths is similar to that of corresponding regions of spontaneously regenerated hydranths (see Table 2). Each part has its characteristic interstitial cell content regardless of whether it is derived from graft or from host tissue.

Interstitial Cell Accumulations and Inducing Activity

Oral cones have dense interstitial cell accumulations in the endoderm. The only other region so far discussed which has this characteristic is the tip of developing outgrowths. Both these regions when cut off and grafted to masses induce the development of basal hydranth regions. There is no other histologically apparent common factor between these two inducing agents: the arrangement of the interstitial cells in the oral cone is not apparent in the outgrowth tip, but in both the interstitial cells are present in large numbers in the endoderm. Whether all tissue with inducing activity has an accumulation of interstitial cells in the endoderm was next investigated.

The regions of the hydroid which have been shown to have inducing action are the oral cone, the tips of outgrowths, the basal hydranth regions and the tentacles (Moore, 1952).

Fig. 7 (plate). The interstitial cell distribution characteristic of other regions than those shown in fig. 6.

A. T.S. stolon tip.
B. T.S. induced outgrowth.
C. T.S. subtentacular region of the hydranth.
D. T.S. subtentacular region of the graft which has produced induction.
E. T.S. grafted tentacle which has produced induction.

Oral cone grafts. Examination of fifty-eight oral cones has established that oral cones have dense interstitial cell accumulations in the endoderm.

Outgrowth tip grafts. Tips of developing outgrowths acquire dense interstitial cell accumulations in the endoderm as development proceeds: a concentration of interstitial cells in the endoderm was found in only one of the

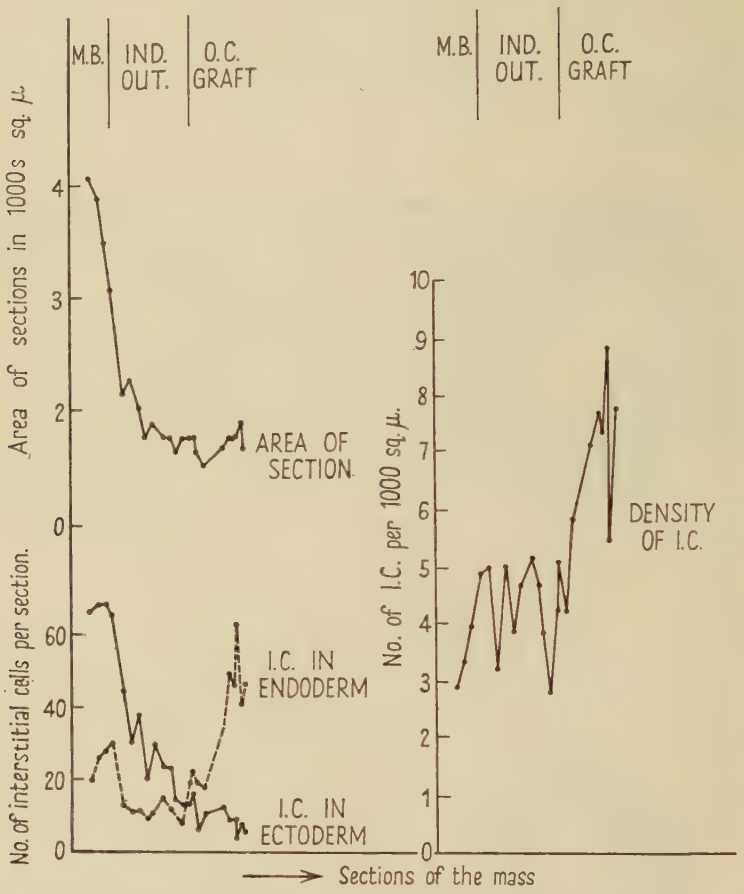

FIG. 8. The interstitial cell (I.C.) distribution and density in a mass with an outgrowth induced by an oral cone (O.C.). IND. OUT., induced outgrowth; M.B., mass body.

ten outgrowths examined from 24-hour-old masses (Table 1, B). Correspondingly, grafted tips of outgrowths from masses only 24 hours old only rarely produce induction (Moore, 1952).

Basal hydranth region grafts. When the tentacular ring, the subtentacular region, or the neck of a hydranth is grafted to a mass, the graft may become reorganized to form the missing apical structures, and induce structures more basal to develop from the mass (Moore, 1952). Since induction is accompanied by the regeneration of an oral cone by the graft, the inducing power of the

raft might be attributed to the presence of an oral cone. Occasionally, however, the appearance of the induced outgrowth precedes the reorganization f the graft, and it is these inductions which provide the test. The subtentacular region was chosen for the investigation, since its interstitial cell ontent is quite unlike that of the oral cone (fig. 7, c).

In four out of forty subtentacular region grafts, induction preceded graft eorganization. These four were fixed before further development occurred, nd examined histologically. In all the grafts examined the appearance had hanged from that of the subtentacular region of the intact hydranth (fig. 7, c). The interstitial cells had become greatly increased in number, and concenrated in the endoderm (fig. 7, d).

The interstitial cell distribution in one of these masses is shown graphically n fig. 9. The interstitial cell densities in the four masses were as follows:

I.C. Densities

Mass body	*Induced outgrowth*	*Graft*	*I.C. accumulated in endoderm*
5–15	11–10	30	+
4–10	10	14	+
7–11	10–17	30	+
3–7	3–7	9	+

These results demonstrate an interstitial cell density peak in the endoderm f the inducing grafts.

Similar results were obtained in a single example of induction by a hydranth eck graft without graft reorganization. Again, in the inducing graft the nterstitial cells were concentrated in the endoderm, which is not the case in he neck of an intact hydranth.

This accumulation of interstitial cells in the endoderm of these grafts may e the first sign of oral cone regeneration by the graft. The fact remains that his is the one histologically apparent characteristic of the oral cone which ppears by the time an induction is produced.

Tentacle grafts. Unlike basal hydranth region grafts, tentacle grafts do not egenerate an oral cone in the course of induction. The whole hydranth, including the oral cone, is induced to develop from the mass (Moore, 1952).

Out of forty-four tentacles grafted, owing to technical difficulty only fourteen became attached to the mass. Two inductions by tentacle grafts were fixed before hydranth differentiation had begun; the grafts had spread out so that the area of section was sufficiently large for the interstitial cell density to be calculated:

I.C. Densities

Mass body	*Induced outgrowth*	*Graft*	*I.C. accumulated in endoderm*
5–10	7–11	26	+
14–20	4–11	28	+

The tentacle grafts showed a dense accumulation of interstitial cells in the endoderm (fig. 7, E), although tentacles in the intact hydranth have few interstitial cells, and then only in the ectoderm.

As was found for the subtentacular region, tentacles when grafted to a mass acquire an interstitial accumulation in the endoderm at the stage of the

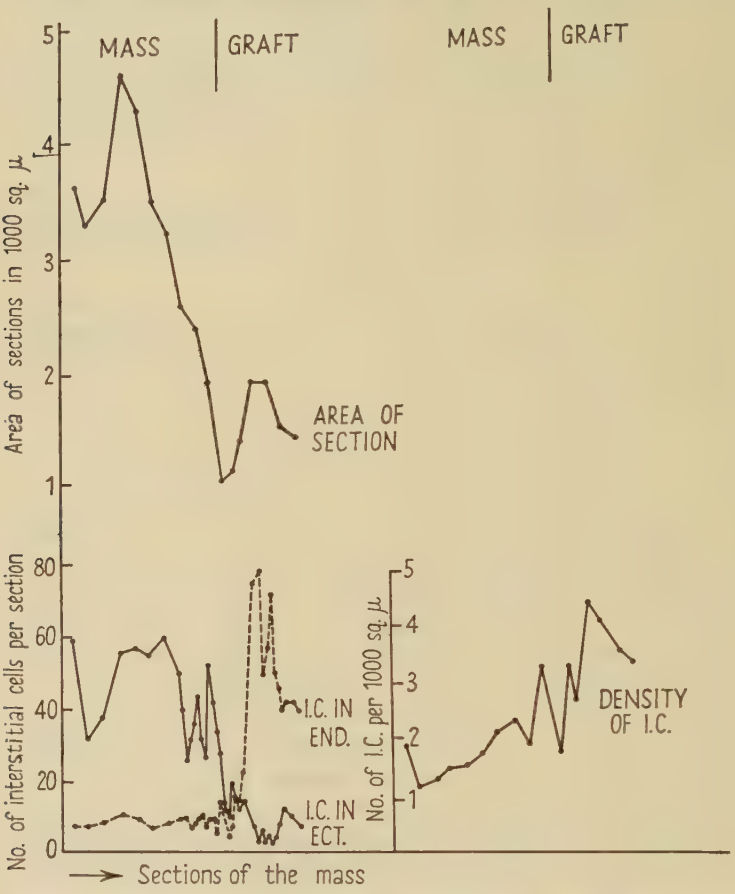

FIG. 9. The interstitial cell (I.C.) distribution and density in a mass with a subtentacular region graft; induction beginning.

appearance of the induction. Further, tentacle grafts do not at any stage in development regenerate an oral cone, so that the appearance of the interstitial cell accumulation cannot in this instance be related to oral cone formation.

Inducing activity is shown by grafts of oral cones, developing outgrowth tips, basal hydranth regions and tentacles. In oral cones and in developing outgrowth tips there are dense accumulations of interstitial cells in the endoderm. When basal hydranth regions and tentacles are grafted to a mass, interstitial cells accumulate in the endoderm of the graft before an induction is produced, although these regions have no interstitial cell accumulation in the

ntact hydranth. Stolon tips and unspecialized pieces of coenosarc have no nducing power, and possess no accumulation of interstitial cells in the endo- erm. The correlation between the function of induction and the structural haracteristic of an interstitial cell accumulation in the endoderm is clearly stablished.

Induction and Ribonucleic Acid Distribution

Evidence exists that some biological inductions, in particular neural induc- ion in the amphibian embryo (Brachet, 1947) may be produced by ribonucleic cid action. In hydranth induction in *Cordylophora* masses an association as been established between interstitial cell accumulations and inducing ower; and since interstitial cells are characterized by their basiphilic cyto- lasm, the possibility of this basiphilia being due to a high concentration of ibonucleic acid was investigated.

Preliminary tests only have been made. Slides of developing *Cordylophora* nasses were incubated with ribonuclease and stained with methyl green yronin (Brachet, 1942). Results were obtained in which the cytoplasm of the nterstitial cells in all regions of the mass was palely stained after incubation vith ribonuclease, but dark in the untreated control, while other cells showed ittle difference in intensity of staining. Further work is required to confirm his indication that interstitial cells are particularly rich in ribonucleic acid, nd, if this is confirmed, to discover the significance of the correlation between nducing activity and an accumulation of cells rich in ribonucleic acid.

Discussion

It has been shown that the tips of developing outgrowths have dense ccumulations of interstitial cells in the endoderm, and that these accumula- ions persist in the adult oral cone. Unlike other tissue, in the outgrowth tip nd the oral cone interstitial cells are not only present in very much greater umbers, but are situated in the endoderm. The interstitial cells are not used p to form the ectoderm and endoderm of the regenerated tissue, and do not ccumulate at sites of growth (stolon tips) or differentiation (induced out- rowths) which are not the sites of oral cone rudiments.

It has also been shown that the regions of the hydroid which induce ydranth development when grafted to a mass—oral cones, developing out- rowth tips, basal hydranth regions and tentacles—all have interstitial cell ccumulations in the endoderm at the time when induction takes place. Basal ydranth regions and tentacles do not have interstitial cell accumulations in he intact hydranth, but acquire them when grafted into a mass. Stolon tips nd unspecialized coenosarc have no inducing action when grafted to a mass, nd have no interstitial cell accumulations in the endoderm.

Interstitial cell accumulations in the endoderm may be directly associated vith the process of induction, or may be an incidental indication of inducing bility. There is some evidence that interstitial cells are particularly rich in ibonucleic acid, but the significance of this cannot yet be assessed.

That the rudiment of the adult oral cone is laid down early in development is in itself suggestive; possibly the oral cone rudiment may control the development of the outgrowth much as an oral cone graft can induce outgrowth and hydranth formation. There is no evidence for 'organizing' activity of the oral cone except under the artificial conditions of grafting, but any findings about oral cone grafts must be referred to the action of the oral cone in normal development. The initiation of a spontaneous outgrowth cannot be ascribed to the action of the oral cone rudiment, however, since the oral cone rudiment is not laid down at the youngest stage of the outgrowth (Table 1, B).

The observed accumulations of interstitial cells in *Cordylophora* might be derived from the local proliferation or from migration of the cells or from a combination of both processes. Material stained with Giemsa's stain is not suitable for accurate mitotic counts, and it can only be recorded that mitoses have been seen in interstitial cells but no intense local proliferations have been found. Evidence that interstitial cells migrate into the tips of developing outgrowths is provided by the relatively low density of interstitial cells in outgrowth bases, and by experiments in which the outgrowth tip was cut off. Further work is required however on this point. The interstitial cells which accumulate in grafts of basal hydranth regions and tentacles can only be derived from multiplication of the few interstitial cells already present in the graft.

Neither the persistence of the interstitial cell accumulations of outgrowth tips in the adult oral cone, nor the correlation between interstitial cell accumulations and induction, has been reported by previous workers. Previous histological work on *Cordylophora* has been done by Kirchner (1934) and Beadle and Booth (1938). Beadle and Booth examined sections of spherical stage masses, using Giemsa's stain, but do not record examination of masses at later stages of development. Kirchner gives a careful account of the functions of interstitial cells in the *Cordylophora* colony, including their part in sexual reproduction and in nematocyst formation, and describes the accumulation of interstitial cells at budding points on the colony. A diagram shows interstitial cell accumulations in the endoderm at the tip of the rudimentary hydranth; no comment is made, and no comparison with the adult hydranth is drawn. Kirchner assumes, as indeed do all previous workers on hydroid regeneration (except Kanajew, 1930, see below), that if interstitial cells accumulate at growing points, their function is to differentiate into the ectoderm and endoderm of the developing hydranth.

Hydra was used as the material in most previous histological work. In hydra there is not an oral cone comparable to that of *Cordylophora*; any accumulation of interstitial cells in the apical structure of the adult might not be observed if interstitial cell accumulation were not the specific object of search. In hydra previous workers have shown that interstitial cells accumulate at the site of buds and regenerants, and yet that new material for buds and regenerants may be formed from already-existing ectoderm and endoderm rather than by differentiation of interstitial cells. The apparent conflict be-

ween these two findings would be resolved if the function of the interstitial ells were not to differentiate into regenerant tissue but to lay down the rudiient of the adult apical structure. Kanajew (1930) found that interstitial cells re essential for the regeneration of the head of the hydra but not for the egeneration of the foot; interstitial cells are required specifically for head ormation, not wherever new material is being regenerated. In *Pennaria*, imilarly, Puckett (1936) showed that when the interstitial cells have been emoved by irradiation hydranths are not regenerated but stolonic growth nay continue.

In the present investigation twenty-one hydras were narcotized with urehane, fixed in Zenker's fluid, sectioned and stained in Giemsa's stain. A horough quantitative investigation of the interstitial cells has not yet been nade, but typically the interstitial cells are concentrated in the ectoderm of he trunk, and no endodermal accumulation in the hypostome is apparent. However, hydras which have been particularly successfully narcotized and ixed with the hypostome extended, show relatively more interstitial cells in he endoderm of the hypostome. Further examination of hydras is required.

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The Innervation of the Skin of Teleost Fishes

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Summary

A detailed study of the innervation of the skin of teleosts by fibres of the general taneous system has demonstrated anatomical differences which may be correlated h the various senses known to be mediated by these nerves. The anatomy of the ixus which these nerves form in the skin is very similar to that of other vertebrates ich have been studied. There exist in the epidermis certain elongated cells, which y be isolated sensory cells taking part in the perception of 'common chemical' muli. It is possible to trace single fibres throughout a considerable part of their arse in the skin; a study has been made of the distribution at the surface of the nches of certain fibres of a type which is almost certainly tactile.

Contents

Introduction

IT has been known for many years that the general cutaneous sensory nerve fibres of teleosts end as free arborizations in the epidermis after rming a plexus in the dermis. Ballowitz (1893), who studied the innervation the chromatophores of perch, pike, and eels, described nerve nets in the rmis at the level of the chromatophore layer and beneath the epidermis, nding sensory fibres into the epidermis where they end freely between the ithelial cells; Eberth and Bunge (1895) also studied melanophore inner- tion and mention the sensory fibres in the dermal plexus. Lenhossék 893), describing taste-buds in the conger eel, figured fibres ending freely the epidermis. Morrill (1895) described a plexus beneath the epidermis of e free fin-rays of *Prionotus*, and showed sensory cells and free endings in e epidermis. Herrick (1901) described the innervation of neuromasts and te-buds in *Ameiurus* and mentioned the free endings without illustrating em or the plexus. Scharrer (1935) and Scharrer, Smith, and Palay (1947) nvestigated the innervation of the skin of the free fin rays of *Trigla* and *ionotus* without entirely agreeing with Morrill.

uarterly Journal of Microscopical Science, Vol. 93, part 3, pp. 289-305, Sept. 1952.]

Studies on the skin innervation of vertebrates other than the bony fis include those of Retzius (1892) on *Petromyzon*, and numerous studies o frogs including those of Eberth and Bunge (1892), Coghill and Herric (1898), Coghill (1899), and Rubin and Syrocki (1936). Not until recently ha much attention been paid to the anatomy of the plexus itself; Wedde (1941*a*) investigated in detail the skin plexus of mammals and also describe in *Acanthias vulgaris*, a plexus of two main layers in the dermis sendin fibres to end freely in the epidermis; he did not report any differentiation o the cutaneous fibres in the fish.

An extensive physiological literature shows that the general cutaneou nerves of teleosts must subserve the tactile, common chemical, temperatur and pain senses. Hoagland (1933*a*) made a direct study of tactile perceptio in *Ameiurus* by recording action currents in the facial nerve. The commo chemical sense of the skin was first definitely separated from the gustator sense by Parker (1908, 1912) and Sheldon (1909) working on *Ameiurus* an *Mustelus canis* respectively; they found the skin sensitive to solutions of acid alkalis, and salts where there were no taste-buds, though there was no re action to quinine except where taste-buds were intact and no reaction t sugar solutions at all. Scharrer, Smith, and Palay (1947) found that thoug such stimuli normally gave rise to avoiding reactions the common chemical sens could, in proper circumstances, be utilized in food-seeking. Bull (1937, 1939 trained fishes to react to small changes in salinity and pH, though his experi ments did not differentiate between the functions of the skin and taste-buds

Wells (1914) and Bull (1928, 1936) investigated the temperature percep tion of teleosts; Wells's experiments are inconclusive as the temperatur 'selection' of his fish may have been due to other causes; Bull used trainin methods and found some fish sensitive to a rise in temperature of as little a 0·03° C. Hoagland (1933*b*) suggested that the lateralis organs might be con cerned with temperature perception (though this had been denied by Parke in 1904) on the grounds that changes in temperature markedly affected th spontaneous discharge in the lateralis nerves; he could obtain no response t temperature change in the spinal nerves. Rubin (1935) found that the re action of fishes to a rising temperature was changed after cutting the mai lateralis branch of the vagus, and concluded that their temperature perceptio had been lost; his experiments are inconclusive for, apart from other objec tions, he had left the head lateralis intact. Sand (1938) recorded impulse from the ampullae of Lorenzini in *Raia* and found them extraordinarily sensi tive to changes in temperature. Dykgraaf (1940) took up the question of th participation of the lateralis system in teleosts and by a series of training experi ments showed that neither the canal organs nor the free neuromasts are con cerned with temperature perception but that, on the contrary, this sense i mediated by the spinal nerves; he was also able to distinguish 'heat' fron 'cold' perception. Pain is mentioned in passing by a number of authors; if is defined as a sensitivity to certain harmful stimuli leading to violent avoidin reactions, its necessity to any animal which is to survive is obvious.

The present study is concerned with the detailed anatomy of the cutaneous nnervation of teleosts, mainly the minnow, *Phoxinus laevis*; methylene blue nd silver impregnation methods were used for the most part.

Methods

The fish were killed quickly by making a cut across the back of the head nd destroying the brain with a needle. If methylene blue was to be used, solution of 0·01 per cent. B.D.H. methylene blue in 1·0 per cent. sodium hloride, made up as recommended by Weddell and others (1940), was njected subcutaneously and the fish left in a shallow dish of saline for from 5 to 45 minutes, according to the structures which it was desired to stain. The skin was then removed from the fish and placed in 8 per cent. aqueous mmonium molybdate for at least 3 hours, often overnight. It appeared to nake no difference whether the ammonium molybdate was chilled or at room emperature. The pieces of skin were cleaned, by dissection in distilled water, f any unwanted tissue such as the argenteum layer, flattened on slides by ying on a glass slip, and taken swiftly up the alcohol series to absolute alcohol. t was necessary to leave the preparation in absolute alcohol long enough to issolve out the pigment of the erythrophores; 2 or 3 minutes were usually ufficient, two baths of alcohol being used. The preparation was cleared in ylene and mounted in neutral Canada balsam. As ethyl alcohol was used uccessfully for the dehydration it was not considered necessary to use the -butyl alcohol series usually recommended.

This methylene blue method could not be used on all parts of the body wing to the close adherence of the skin to the muscles in some regions; in the innow the most favourable areas were the opercula and cheeks, and the egion between the throat and pelvic fins, which, in all except the largest pecimens, is almost devoid of scales. In the stickleback only those parts of he skin which are free from scutes can be prepared. Another disadvantage f the methylene blue method is that the staining in the epidermis is never of igh quality and that the epidermis is always loosened and damaged to some xtent owing to the lack of proper fixation. Rongalit methylene blue was used, ut found less satisfactory than the unreduced dye. Dissected pieces of skin ere sometimes immersed in the unreduced dye to stain the lateralis system, ut the quality of the staining in the general cutaneous nerves was then poor.

Owing to the lack of proper fixation in the methylene blue material it was ecessary to find a silver method which could be used alternatively. For araffin sections the method of Holmes (1943) was successful to an extent hich depended on the fixative (see details below); this method can also be sed for pieces of skin provided they are first dehydrated and treated with ylene, but is not then of much value owing to lack of contrast between the bres and the surrounding tissues of the thick preparation. After experiments ith ammoniated silver solutions and a variety of fixatives the following outine was adopted: the fishes were left in a bath of the appropriate fixative r several days, then skinned, and the skin left for some hours in distilled

water before treatment overnight in 10 per cent. silver nitrate in the dark. The impregnating bath was of a 0·5 per cent. or 1·0 per cent. solution of ammoniacal silver hydroxide, prepared either with drops of 25 per cent. sodium hydroxide and strong ammonia, or with the balanced solutions recommended by Kubie and Davidson (1928). In the latter case 1·2 M ammonia from a burette was added to 5·0 ml. of 0·6 M silver nitrate in a 100 ml. volumetric flask until the precipitate just dissolved; either 5·0 ml. or 2·0 ml. of 0·6 M sodium hydroxide was added and more ammonia until the solution cleared again; as it was important to add no excess of ammonia, a few granules of precipitate were left in the flask; the volume was then made up to 100 ml. with distilled water. The pieces of skin were washed in two changes of distilled water before being treated in the silver bath for 5 minutes. After washing again in two changes of distilled water they were passed to a hydroquinone-sodium sulphite reducing solution, which was that used in Holmes's method diluted by half, then washed again, and examined. If the nerve impregnation was faint the specimens were toned with gold in the usual way, but if the silver impregnation was distinct it was preferable to omit the gold toning which darkened the background.

Only three of the number of fixatives used were found to be of value. The most useful, and the only one after which it was possible to trace the epidermal fibres adequately was:

Glacial acetic acid	5 ml.
Absolute alcohol	95 ml.

This mixture is mentioned by Bodian (1937). An extensive impregnation can be obtained; the mucous cells and the nuclei of the epidermal cells are usually heavily impregnated, but as the background is otherwise fairly clear, it is possible to trace fibres in spite of this disadvantage. Another reliable fixative used by Holmes (1943), consists of:

Glacial acetic acid	5 ml.
Picric acid, saturated solution in 0·5 per cent. saline.	95 ml.

The chief disadvantage in this case is that the epidermis shrinks away from the dermis and becomes torn over the scales. A mixture of:

Ammoniated 20 per cent. formalin . .	30 ml.
Absolute alcohol	70 ml.

used by Willis (1945) occasionally produced excellent results, especially for the fine fibres, but was less reliable. It was necessary to leave the fish in the fixative for some weeks and then to wash in 70 per cent. alcohol for a further period of about two weeks before impregnating; gold toning was essential. The mucous cells were not heavily impregnated, but the nuclei of the dermal and epidermal cells were dark; epidermal fibres could not be traced. A variety of other formaldehyde fixatives was used, all without success, nor was chloral hydrate of use.

With Holmes's method using dilute solutions of buffered silver nitrate, dermal fibres could be demonstrated in sections from acetic/alcohol terial at a pH of 7·8 and in picric/acetic material at pH 7·4 (there was a ft to pH 7·6 on addition of the silver); in both cases there was a silver rate concentration of 1 in 10,000 and the sections were incubated over-ht.

These silver methods cannot be used to estimate the diameters of the fibres; apart from the fact that they are primarily axon stains, the apparent calibre the fibre depends on the amount of silver deposited, which is variable. ost of the silver material was obtained from minnows; the same ammoniacal ver method was successful for *Clupea sprattus* and *Esox lucius*, though not ccessful for *Ammodytes* or *Perca*. In addition to the above methods some mium preparations were made; the fish were fixed in 20 per cent. formalin in formaldehyde-saline, and the dissected skins left for some 20 hours in per cent. osmium tetroxide; this length of time was necessary as the solu-n did not readily penetrate into the dermis.

The Anatomy of the Plexus

In the minnow the skin plexus is best seen in the area between the throat d pelvic fins, where it is uncomplicated by scales or lateralis nerves. On ching the skin the nerves form a sub-dermal plexus lying in the deepest er of the dermal connective tissue, which may also be the argenteum and deep melanophore layer; this plexus is connected by discrete vertical ndles to the sub-epidermal plexus, which lies in the upper layers of the rmis and immediately below the epidermis. Fibres leave this plexus to netrate the basal layer of the epidermis and to ramify in the epithelium. ough the sub-dermal plexus is continuous, the segmental nature of the rve supply is here apparent, while in the sub-epidermal plexus, which is of aller nerve bundles and finer mesh, it is not. Individual fibres branch at th levels, and any bundle may contain fibres running in either direction. casionally fibres have been seen to descend again from the sub-epidermal the sub-dermal level. It is common for a fibre to leave the main part of sub-epidermal plexus and to run for a little distance immediately below epidermis, then to descend again with or without sending a branch into epidermis. A section of the skin showing a vertical bundle and a portion the sub-epidermal plexus is seen in fig. 1, A; the sub-dermal plexus is here scured by the overlapping of the muscles; it is characteristic that many of fibres in the epidermis run parallel to the surface for some distance after y have passed the basal layer.

Lateralis fibres do not usually enter the cutaneous plexus proper, the bundles the free neuromasts remaining separate. The communis nerves to the taste-ds on the surface of the head and body enter the plexus separately but do m part of it; it is usual for the bulk of the nerve supply to a taste-bud to end from the sub-dermal level as a discrete bundle, but other communis res run in from the sub-epidermal plexus. A number of general cutaneous

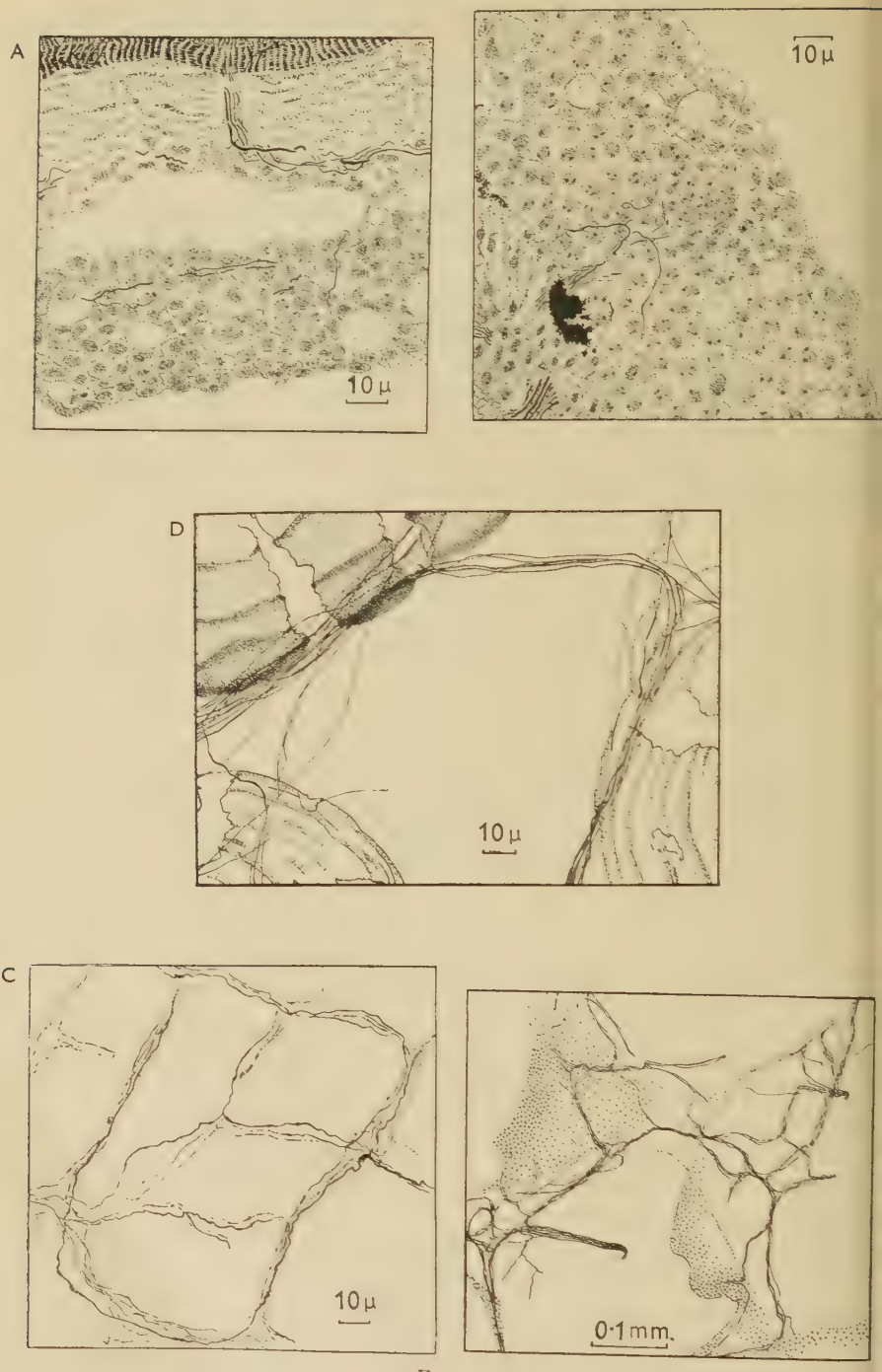

FIG. 1

ores always pass into the dermal papilla of the taste-bud, but then leave to end in the surrounding epithelium in the usual way; similar fibres *Conger* are figured by Lenhossék. The existence of these fibres may count for the observation by Herrick (1901) that the fibres in the dermal papillae of the taste-buds on the body of *Ameiurus* are larger and more thickly myelinated than those usually encountered in the communis nerves. This arrangement, which is shown in fig. 1, B, makes it difficult to distinguish communis fibres from the general cutaneous, unless the collaterals can also be traced to dermal papillae.

A portion of the sub-epidermal plexus from a scaleless region of the chest, as seen in whole mount, is shown in fig. 1, C. The presence of scales complicates the form of the sub-epidermal plexus, as the more superficial, posterior half of the scale lies at this level and the fibres must pass around it. In a young minnow the scales are small and lie far apart, not interfering with the general form of the plexus (see fig. 1, D); but as the scales grow and come to overlap, their advancing edges distort the nerve plexus, breaking it up into parts lying in the posterior halves of the dermal scale pockets, though connected with those parts in adjacent pockets. From here fibres pass either up to the outer surface of the same scale or back above the scale behind; in either case they eventually pass into the epidermis in the usual way. The arrangement of the sub-epidermal plexus on the flank of a mature minnow is shown in fig. 1, E, though here the upper portion was lost when the scales were removed.

The fibres which compose the plexus may be myelinated or unmyelinated and are of various calibres. Usually each bundle contains a few fibres which are noticeably larger than the rest. These can be seen in figs 1, C, 3, A, and 3, B, which are methylene blue preparations, and in fig. 2 where they are impregnated with osmium tetroxide. The diameter of a fibre decreases as it branches; the diameter of one of the coarser fibres, measured in an osmium preparation at the sub-dermal level, decreased from $5{\cdot}5\,\mu$ to $2{\cdot}7\,\mu$; the usual figure obtained for similar fibres in the sub-epidermal plexus was $2\,\mu$ or a little under. The diameters of the finer myelinated fibres in the sub-epidermal plexus were

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FIG. 1. Minnow: camera lucida drawings. A. Transverse section of the skin near the pectoral fins; the epidermis, which shows mucous cells and fragments of epidermal fibres, has become detached from the dermis, which contains a rising bundle and portions of the sub-epidermal plexus. Holmes's method, fixed acetic/alcohol. B. Transverse section of head, showing a taste-bud with general cutaneous fibres rising in the dermal papilla and passing out into the epidermis. A portion of the sub-dermal plexus is seen in the lower left-hand corner. Holmes's method, fixed acetic/alcohol. C. Whole mount of dermis of chest, showing a portion of the sub-epidermal plexus. One bundle rises from the sub-dermal level and three pass into the epidermis. Methylene blue. D. Whole mount of dermis of skin of flank, showing the sub-epidermal plexus where the scales do not overlap. The upper left-hand bundle passes under the posterior edge of a scale, the others pass over the anterior edges of scales. Ammoniacal silver hydroxide, toned gold chloride, fixed ammoniated formol/alcohol. E. Whole mount of dermis of skin of flank with scales removed, showing the sub-epidermal plexus where the scales do overlap (contrast D). The stippled areas represent portions of the epidermis not removed with the scales. Rising bundles pass from right to left, forming plexuses in the posterior halves of the scale-pockets. Ammoniacal silver hydroxide, toned gold chloride, fixed acetic/alcohol.

estimated as between 1 and 1·5 μ; these included the communis fibres. There was difficulty in determining the diameters of such small fibres, owing to the errors of measurement; the finest unmyelinated fibres present were near the limit of visibility even under the greatest resolution available.

The size of the mesh of the sub-epidermal plexus was approximately the same in both large and small minnows. Usually the coarser fibres branched at longer intervals than the finer ones and consequently did not enter into all the bundles, the lumen of a mesh containing coarse fibres being crossed by

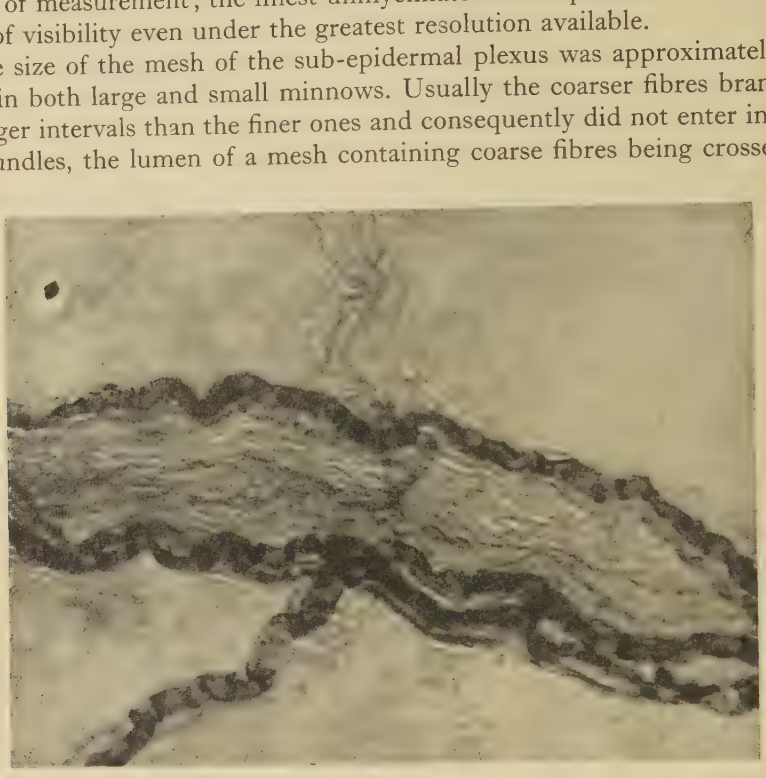

Fig. 2. Minnow: photomicrograph. Whole mount of dermis of chest showing myelinated fibres in a bundle of the sub-dermal plexus. Osmium tetroxide. ×465.

bundles of finer fibres (fig. 1, c). It is assumed that as growth proceeds and the plexus is stretched in all directions branches of the coarse fibres will eventually penetrate the finer bundles and that new bundles will be put out to cross the enlarging meshes. Some fibres in any case leave the nerve bundles to run between them in the dermis; these are extremely fine and are apparently unmyelinated (fig. 3, b); they run mostly in the outer part of the dermis, though not all in one plane; it was not certain if they also entered the epidermis; they may have done so, but were too fine to be traced there.

Most of the fibres left the sub-epidermal plexus to pass through the basal layer of the epidermis, apparently at any point; the myelin sheath was lost as the fibre entered the epithelium. In the epidermis the fibres continued to branch, ramifying between the epithelial cells. Only the coarser fibres could be adequately traced in the epidermis, where they branched and ramified throughout its thickness; some branches approached the surface directly, others ran horizontally or ascended and descended again; eventually they

ıme to free endings. Some branches ended immediately below the cuticle hich covers the outer surface of the epithelium, though other branches of ıe same fibre might end apparently quite deep in the epidermis; there was ways a doubt in such cases as to whether the fibre had really ended or whether ıe impregnation had ceased. The finer fibres could not be followed with rtainty in the epidermis as they were not well impregnated with silver and nded to be beaded in the methylene blue preparations; those fine myeli- ated fibres which were not communis appeared to branch repeatedly just ove the basal layer of the epidermis, but it was not certain whether some any of them approached the surface.

The nerve supply of the cornea of the minnow consists of a circumferential ericorneal plexus (see Zander and Weddell, 1951) from which arise bundles assing into the cornea radially, but soon branching in all directions and rming a plexus similar to that seen elsewhere on the body, though most of ıe fibres run radially; individual fibres commonly pass across the centre of ıe cornea into the opposite side. The radial bundles enter the cornea at two vels, between the scleral and dermal, and the dermal and epidermal layers; ıere is not a clear distinction between these plexuses as they are connected y rising bundles and in the central part of the cornea can scarcely be separ- ed. Bundles of fibres pass into the epidermal layer as elsewhere on the body; bres were seen to run extensively in the lower epidermal layers, but it was ot certain whether or not they penetrated the superficial layers of flattened lls. Only silver preparations of the cornea were made, but as far as could e judged both coarse and fine fibres were present.

Nerve bundles run into the fins not only within the hollows of the fin rays ut also in the web; groups of fibres pass out of the fin rays at regular intervals etween the lepidotrichia. The arrangement of the nerve bundles in the fins not entirely radial but plexiform in that there are cross connexions between undles.

The distribution of the fibres in both dermis and epidermis appeared to be ndom in that no precise pattern was discernible, nor were they spaced venly. The form of the plexus was presumably governed by the form of the rrounding tissues when innervation took place. No evidence of fusion of bres into a continuous nerve-net was seen, except in one doubtful case; rtifacts giving this appearance may easily arise, especially with silver methods.

The motor fibres of the melanophores also run in the skin plexus, and could e detected in any of the better silver preparations of the minnow, though ıere was often considerable practical difficulty in identifying them; the ze of the mesh of the plexus and of the melanophores is such that at ıe sub-epidermal level each melanophore must overlap a nerve bundle, nd is very likely to do so at the deeper levels. Melanophores may become ctually applied to the sensory nerve bundles. The innervation of teleost ıromatophores was described by Ballowitz (1893) and Eberth and Bunge 1895); the condition in the minnow agrees in all respects with their descrip- on of other species. A melanophore fibre of a minnow is shown in fig. 3, c:

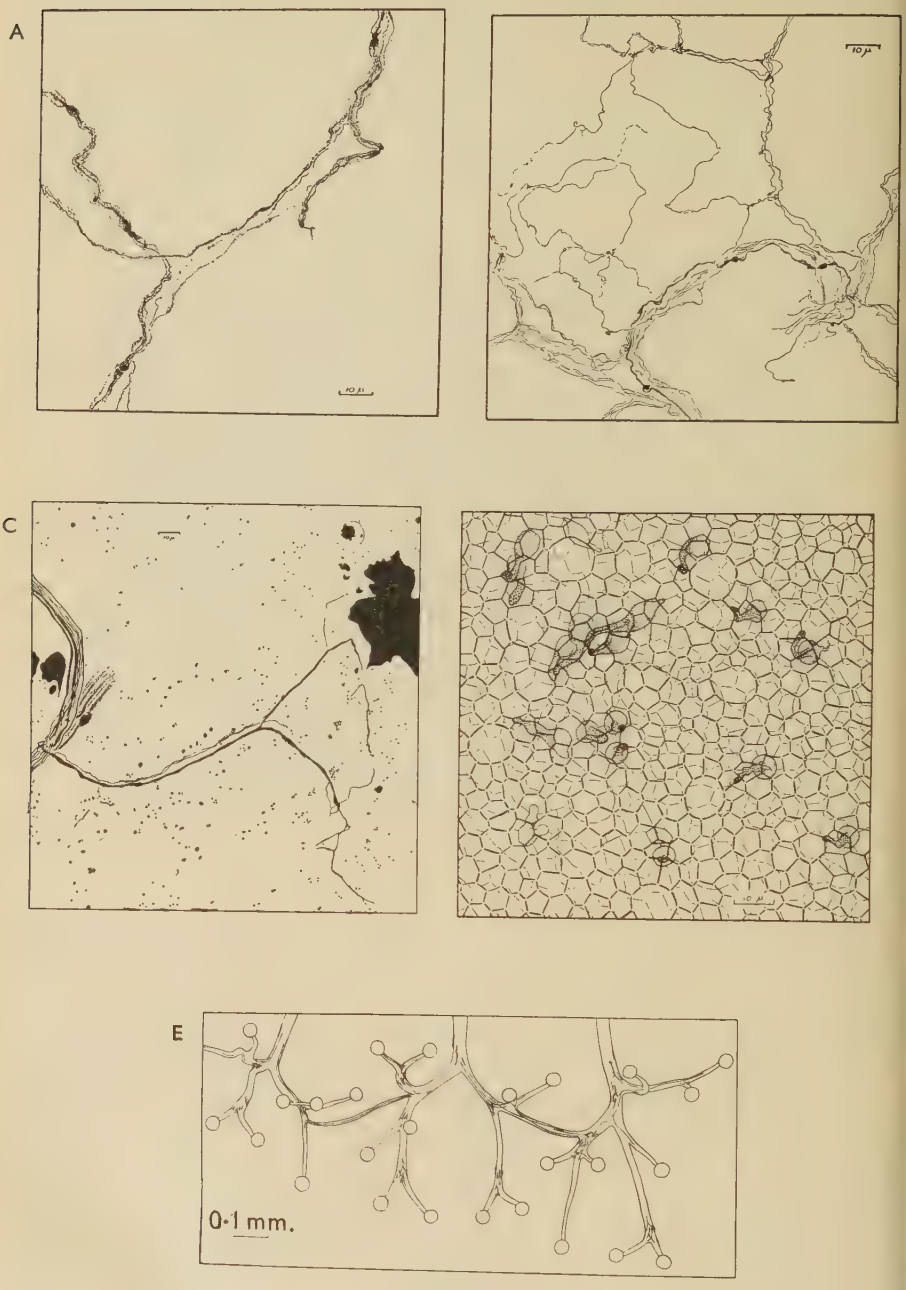

Fig. 3

he melanophore in this case was lying under a scale in the anterior part of scale-pocket, so that the area is free of sub-epidermal bundles; there is no way of telling whether the fine fibres accompanying the coarse one are also notor, or whether they are the dermal sensory fibres; the area is covered by he branches of three melanophores, which are contracted, though numerous granules of melanin which have remained in the arms may be seen. The branching of the melanophore fibres may occur at some distance from the melanophore itself, the various branches being distributed in all directions; he branches do not necessarily end on the melanophore but may pass on to upply others.

The anatomy of the plexus in the other fish studied was essentially similar o that in the minnow. Silver preparations were made of *Clupea sprattus*, nd methylene blue preparations of *Gobio*, *Gasterosteus*, *Ameiurus*, and *Esox*.

Isolated 'Sensory' Cells in the Epidermis

Morrill (1895) reported the presence of isolated sensory cells in the epidermis of the free fin rays of *Prionotus*, which he demonstrated by gold chloride, methylene blue, and Golgi methods. Coghill (1899) described similar cells on he head of the frog. Later authors seem to have neglected these observations; Scharrer, Smith, and Palay (1947) were not able to find such cells in *Trigla* or *Prionotus*.

In suitable methylene blue preparations of the minnow certain cells in the epidermis stain a bright blue, though the general epithelial cells and the mucous ells are uncoloured. These cells are flask-shaped or spindle-shaped, the part which contains the nucleus being about the same size as an epithelial cell; process ascends towards the surface and usually a smaller one projects into he deeper layers of the epidermis. The cells lie in the epidermis at various evels, but are sometimes disorientated by loosening of the epidermis during preparation. There is often a swelling at the end of the outer process which may have the appearance of a secretion, but this is part of the cell and lies within the epithelium; Morrill shows similar swellings in illustrations drawn rom gold chloride material. The cells have the same appearance as those which stain blue within the taste-buds; they frequently lie in groups of three or four like small taste-buds, but it is quite certain from the configuration of

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Fig. 3. Minnow: camera lucida drawings. A. Whole mount of dermis of chest, showing nyelinated fibres of the sub-epidermal plexus, with two fibres rising to the epidermis. Methylene blue. B. Whole mount of dermis of chest showing the sub-epidermal plexus as een when the finer fibres are stained (contrast A). The bundle in the bottom right-hand corner s rising in the dermal papilla of a taste-bud. Methylene blue. C. Whole mount of dermis of lank with scales removed, showing a melanophore fibre. The bundle on the left is part of he sub-epidermal plexus of the scale-pocket; the bulk of the melanophore on the right lies n a lower plane than the fibre and the arm which it innervates. Ammoniacal silver hydroxide, oned gold chloride, fixed acetic/alcohol. D. Whole mount of skin of chest in surface view, howing the vertically elongated spindle-shaped cells lying in the epidermis; the epithelial ells are drawn diagrammatically. Methylene blue. E. Whole mount of operculum, showing he nerve supply to certain free neuromasts. The positions of fibre dichotomies are indicated. Methylene blue.

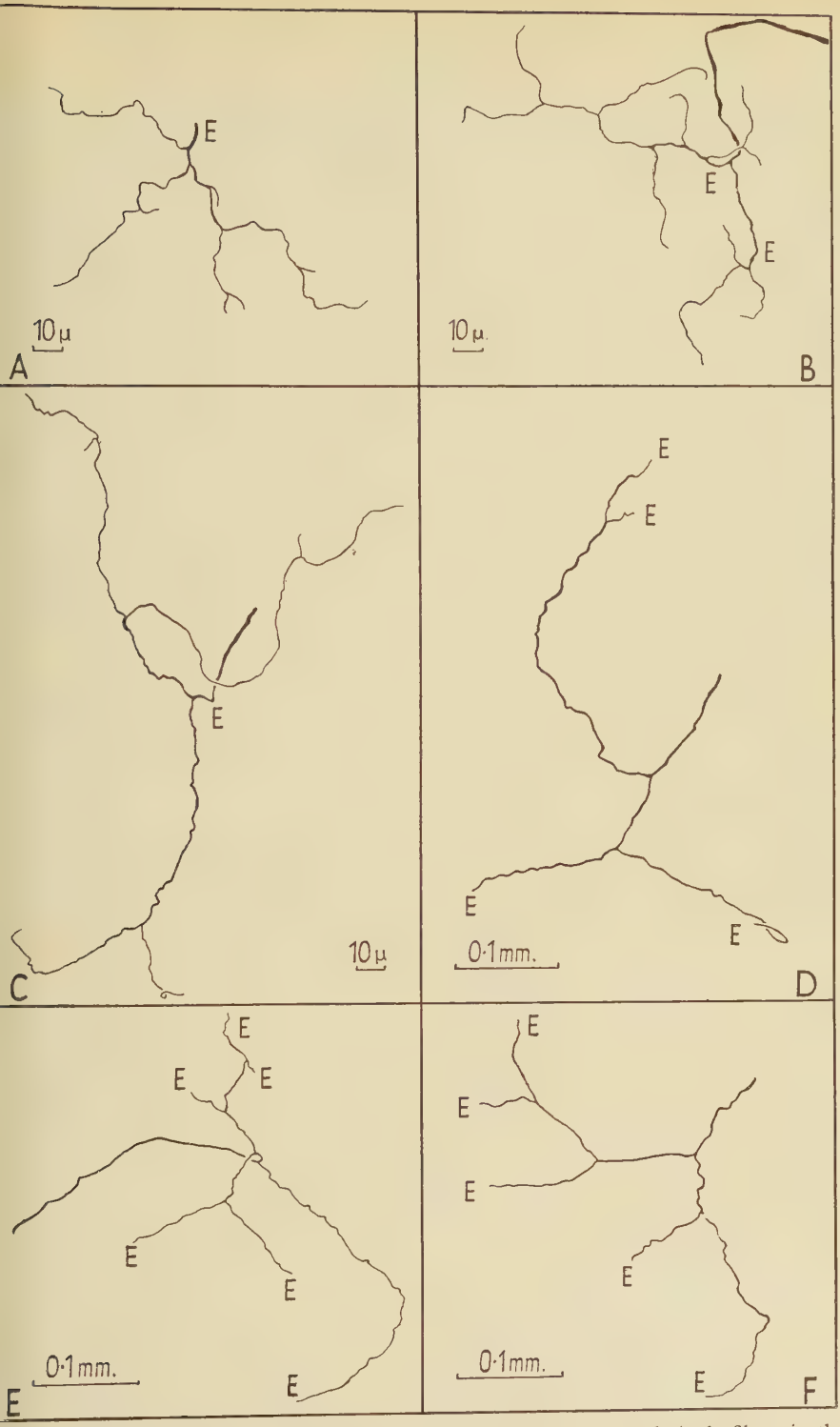
the surrounding epidermis that these are not taste-buds. Fig. 3, D shows the cells as seen in surface view of a whole mount; the epithelial cells are drawn diagrammatically for convenience. Similar cells have been identified in the epidermis of *Gasterosteus*, *Ameiurus*, and *Esox*.

The fact that these cells stain with methylene blue is not in itself an indication of a nervous nature, for non-nervous cells such as the erythrocytes will stain in appropriate circumstances. The goblet cells and epithelial cells, however, never stain under these conditions. A definite nervous connexion is difficult to demonstrate because the fibres do not reach their point of optimum staining until after the cells have faded. It is certain that the coarsest medullated fibres do not innervate these cells, for they have been seen to end freely in the superficial layers of the epidermis; also the distribution of their branches is too sparse. In some cases branches from the finer medullated fibres have been seen to lie against the proximal processes of the cells; this is not proof that they are in actual connexion, as the fibres may be encountered anywhere between the epidermal cells and unless all the collaterals could also be traced the question cannot be settled; this it has not been possible to do. The possibility that the cells were secretory and innervated would still remain, but in that case the nature of their secretion would have to be settled. They are quite distinct from the ordinary mucous cells of the epidermis which are of a different size and shape and which have never been seen to stain with methylene blue. There are in the skin of certain fishes other secretory cells, the club cells or thread cells, which secrete a slime that is not mucus (Reid, 1894); these do not occur in the minnow, but are present in the pike, where the spindle-shaped cells have also been identified and are distinct. The cuticle which covers the surface of the epithelium of teleosts is secreted by the general epithelial cells. There is no reason to assume that the elongated blue cells should be supporting cells; connective tissue cells have been reported from the epidermis (Reid describes them in the eel), but these are fibroblasts which wander in from the dermis. The likelihood is therefore that the spindle-shaped cells are sensory.

The elongated cells could not be identified in the silver preparations, for these methods do not demonstrate cell boundaries; their exact relationship to the surface therefore remains in doubt. In some methylene blue preparations of the minnow and catfish, where the surface of the epithelium was apparently intact, the outer process of the spindle-cell reached the surface between the corners of the epithelial cells.

The Peripheral Distribution of Single Fibres

The distribution within the epidermis of fibres on the chest (fig. 4, B) and operculum (fig. 4, A, C) of a minnow of length 5 cm. is shown; the fibres in each case enter the epidermis at the point (or points) marked 'E'. There is no certainty that the fibres are impregnated as far as their true endings, but if the fibres are excitable throughout their course in the epidermis (the myelin sheath does not extend beyond the dermis) the exact distribution of the



IG. 4. Minnow: camera lucida drawings. A, B, and C. The course of single fibres in the pidermis; the fibres enter the epidermis at the points marked 'E'. Ammoniacal silver ydroxide, fixed acetic/alcohol. D, E, and F. The course of single fibres in the sub-epidermal plexus; the fibres pass into the epidermis at the points marked 'E'. Methylene blue.

endings proper is of less importance than it would be if only the tip of the axon were functional as a receptor. The epidermal spread of twelve fibres was plotted by means of camera lucida drawings; the greatest distance observed between any two endings of a fibre was 0·25 mm., and the mean of the greatest distances in the twelve fibres was found to be 0·14 mm.

The spread of a branch of a fibre in the epidermis is small compared with its distribution in the dermis. Fig. 4, D, E, and F show the courses of single fibres in the sub-epidermal plexus up to the points at which they entered the epidermis ('E'). All are coarse medullated fibres in the ventral skin of minnows. The sub-epidermal spread of eight of these fibres was plotted, four from a single fish, the others each from a different fish of approximately the same size. The greatest distance between any two points at which a fibre entered the epidermis was 0·61 mm., while the mean of the greatest distances in the eight cases was 0·39 mm.; on this should be superimposed not only the epidermal spread but also a sub-dermal spread; the latter unfortunately could not be measured owing to the difficulty of following single fibres through the large nerve bundles of the sub-dermal plexus. The nature of the plexus is such that the sub-dermal spread need not necessarily exceed that at the sub-epidermal level.

The actual spread of single fibres may vary widely in a single individual; no constancy of pattern is discernible, as if the factors influencing the distribution of the fibres in the skin were purely local. The lack of a recognizable pattern makes the comparison of the innervation of any particular area in fishes of different sizes a matter of difficulty. A proportion of the fibres in the lateralis nerves to the free neuromasts is found to branch, each fibre supplying a group of neuromasts which are descended from a single one in the young fish; the number of free neuromasts increases as the fish grows, but their innervation can only take place from the fibres already present. Fig. 3, E records the number and position of fibre dichotomies seen in a methylene blue preparation of free neuromasts on the operculum of a minnow; as only some of the fibres present take the stain no attempt was made to estimate the proportion of branched to unbranched fibres. The spread of such a lateralis fibre will depend on the increase in the number of neuromasts since their definitive innervation took place. Similarly, the spread of a general cutaneous fibre will depend on the growth of the fish; the sensory areas set up at the time of the definitive innervation of the skin may be compared to the dermatomes, which are equal to the sum of the sensory areas of each segment and which also must increase in size as the animal grows. To relate the spread of a fibre to the size of the fish, it may be said that in the minnow and also in the gudgeon a single fibre will spread over at least the area of one scale and will frequently spread to an adjacent scale as well; this can be seen by comparing fig. 1, E with fig. 4, D, E, or F, but has also been verified by direct observation; the same fibres will remain in relationship to a scale throughout its growth. The distance of two-point discrimination cannot be less than the spread of the fibres concerned, so that the fish cannot, presumably, discriminate between one scale and the next, but only between that and the next but one.

In man the distance between touch-spots is said to be 0·1 mm. on the finger tips, 0·4 mm. on the back, and 0·6 mm. on the upper arm; if in a minnow the branches of a single tactile fibre extend to 0·6 mm. apart, its two-point discrimination must be less acute than that of man, in spite of the difference in the size of the animals. In the frog the surface spread of a single tactile fibre is said to be from 4 to 100 sq. mm. (Adrian, Cattell, and Hoagland, 1931). In the fish as in man the area supplied by one cutaneous fibre also receives other fibres of the same type, so that there is a multiple innervation of sensory spots (or areas), an arrangement well known to be characteristic of skin nerves (Tower, 1942). The multiple innervation cannot here have the same significance as that suggested by Weddell (1941*b*) for Meissner's corpuscles, that is, that it is a mechanism for the precise localization of stimuli, for the fibres to Meissner's corpuscles are stated specifically not to branch in the dermis, while all the fibres seen in the fish do branch, the collaterals coming into association with the branches of various other fibres. Weddell states that other fibres in man do branch, for instance those leading to Krause's end-bulbs; the fact that the fibres to Meissner's corpuscles do not branch is in itself a mechanism for the precise localization of touch.

The fish skin might be compared with the cornea of a mammal, which has been extensively investigated; the literature is reviewed by Zander and Weddell (1951). Tower (1935) recorded impulses in the ciliary nerves of cats and concluded that the sensory ending in the cornea consists of all the terminal tissue of a nerve fibre, which is a unit, activity in any part of which influences the whole. The total corneal sensory mechanism is an aggregate of overlapping units which are physiologically independent. Apart from the fact that in the fish skin there are several sense modalities while in the cornea there are only two (pain and a variety of touch), such a description could be applied to the fish skin, for the anatomical arrangement of the plexuses is very similar.

Discussion of Function

It has been seen that the anatomical differentiation of the nerve endings in the fish skin, which must reflect undoubted physiological differences, is not very strongly marked, yet can be detected. Any assignment of function can only be speculative, but there is a certain amount of evidence available on the physiological side both in fish and, more fully, in the frog.

Adrian, Cattell, and Hoagland (1931) were able to isolate impulses from single fibres in frogs by recording antidromic impulses; tactile stimulation by a jet of air gave rise to large fast waves of a type presumed to be carried by the larger medullated fibres; the application of weak acid to the skin initiated a discharge of small, slow waves, as did stimulation of the dermis, by scraping, after the epidermis had been removed. Hoagland (1932) showed that stroking frog skin and applying filter paper soaked in acetic acid produced bursts of impulses from approximately the same area; the response to temperature was surprising in that there was no discharge on moderate warming, while considerable heat produced a discharge in only three fibres out of some fifty

in the nerve concerned; the response might be rapid or slow, but was generally slow. Morgan (1922) found that treatment of spinal frogs with cocaine eliminated first touch, then cold, then heat, and that reactions to pain and to acid remained last.

Hoagland (1933*a*) recorded action potentials in the facial nerve of *Ameiurus*; mechanical stimulation of the skin produced bursts of rapid impulses of large amplitude. Chemical stimuli gave rise to rather indistinct impulses of low potential; evidently the recording apparatus was not sufficiently sensitive. The solutions used as stimulants were of acetic acid, sodium chloride, meat juice, and sugar, of which the last had no effect, as would be expected from Parker's experiments on the behaviour of the fish. Hoagland did not differentiate between the gustatory and the common chemical senses, both of which are mediated by the facial nerve; both meat juice and acetic acid gave rise to slow waves, indicating the activity of small fibres in each case. Hoagland was not able to detect impulses arising from temperature stimuli in the spinal or facial nerves (1933*b*).

On the basis of this physiological evidence it appears that the coarse medullated fibres so conspicuous in the plexus are tactile. The status of the remaining types of fibre is less clear, but by analogy with the frog experiments the fine, apparently unmedullated fibres in the dermis (fig. 3,B) might be expected to be pain fibres; the fact that in mammals some painful stimuli are transmitted by medullated fibres need be no deterrent, for in mammals there are apparently two types of pain transmission, fast and slow (see discussion in Tower, 1942). The unmedullated fibres can presumably be stimulated anywhere along their course by damage or by substances released from the surrounding tissues when they are damaged.

The fibres of the gustatory and common chemical senses are known to be fine; those fibres apparently associated with the spindle-shaped cells of the epidermis are of the same calibre as the communis fibres. As the other chemical senses, taste and smell, are mediated by epidermal sensory cells, it might be expected that sensory cells are concerned also with the common chemical sense. Parker (1912) assumed that the nerve endings for the common chemical sense were free, but mentioned Botezat's suggestion that the stimulus was transmitted by substances produced in the epidermal cells by the action of the external solution. Crozier (1916) states that the stimulants used in investigating the common chemical sense do not penetrate into the epidermis, so that it is unlikely that there is direct stimulation of the axon in the epidermis. Scharrer (1935) suggested that the goblet cells might also act as sensory cells, but later (1947) withdrew this opinion. It is to be presumed, although there is no anatomical evidence, that there is more than one type of chemoreceptor. There is no need to assume the existence of a large number of different chemoreceptors: there is evidence that, for example, in all acids it is the hydrogen ions which actually provide the stimulus (see Sheldon, 1909; Cole and Allison, 1933).

Owing to the difficulty of making accurate measurements it was not possible to study in detail the range of fibre diameter, but fibres of a diameter less than

the tactile and greater than the communis were seen; of the five fibres stained in fig. 3, A the two largest are of the 'tactile' calibre, while the smallest is of the 'gustatory' size, though it may not be communis; possibly the remaining fibres serve a temperature sense. Many more small fibres must be present, but are not stained in this preparation. It should be emphasized that the diameter of a fibre can never be taken as an absolute criterion of its function, though it may serve as an indication. The fate, in the epidermis, of the fibres of intermediate diameter was uncertain.

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Internode Length in the Skin Plexuses of Fish and the Frog

By MARY WHITEAR

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Summary

Measurements made in the skin plexuses of teleosts and of *Rana* have shown that he internode lengths of myelinated fibres may here be shorter than the 'minimum nternodal length' previously reported.

INVESTIGATIONS into the anatomy of vertebrate peripheral nerves have included studies of the spacing of the nodes of Ranvier on medullated fibres of various diameters; there is a relationship between internode ength and fibre diameter, which was shown by Vizoso and Young (1948) to depend on the time of medullation of the fibre and its subsequent growth. Several workers, whose results are summarized by Thomas and Young (1949), reported that the length of the myelin segment between adjacent nodes has a minimum value, regardless of diameter; this figure, which may be of importance in a consideration of the physical properties of the myelin, is usually given as 200 μ, though Young (1945) records it as 150 μ in the foetal rabbit. The diameters of the fibres concerned were not less than 2 μ; studies were made on mammals, amphibians, and fishes.

During an investigation of the anatomy of the skin plexus of the minnow, *Phoxinus laevis* (Whitear, 1953), it was noticed that the myelin segments were considerably shorter than any previously reported, being about 50–60 μ in ength. The skin was stained by the local subcutaneous injection of methylene blue and subsequently treated with ammonium molybdate, dehydrated and cleared in the usual way. Measurements of internode length and fibre diameter were made with a moving-wire micrometer eyepiece, under the highest convenient magnification. The fibres in the plexus are seldom straight, so that some errors in the measurement of length were unavoidable, although measurements were not made on the more tortuous fibres. To determine the extent of the error, measurements of 28 internodes, on four fibres, were made twice, independently, with the $\frac{1}{8}$-inch objective. The mean of the differences between the 28 pairs of measurements was 0·7 μ with a standard error of $\pm$0·5 μ ($t = 1{\cdot}40$ for 26 degrees of freedom); it follows that there was no significant difference between the two sets of measurements. (Another source of error lies in shrinkage during preparation. Since the fibres cannot be observed until the material has been cleared this error is not measurable, but can be assumed both to be constant and to be insufficient to account for the

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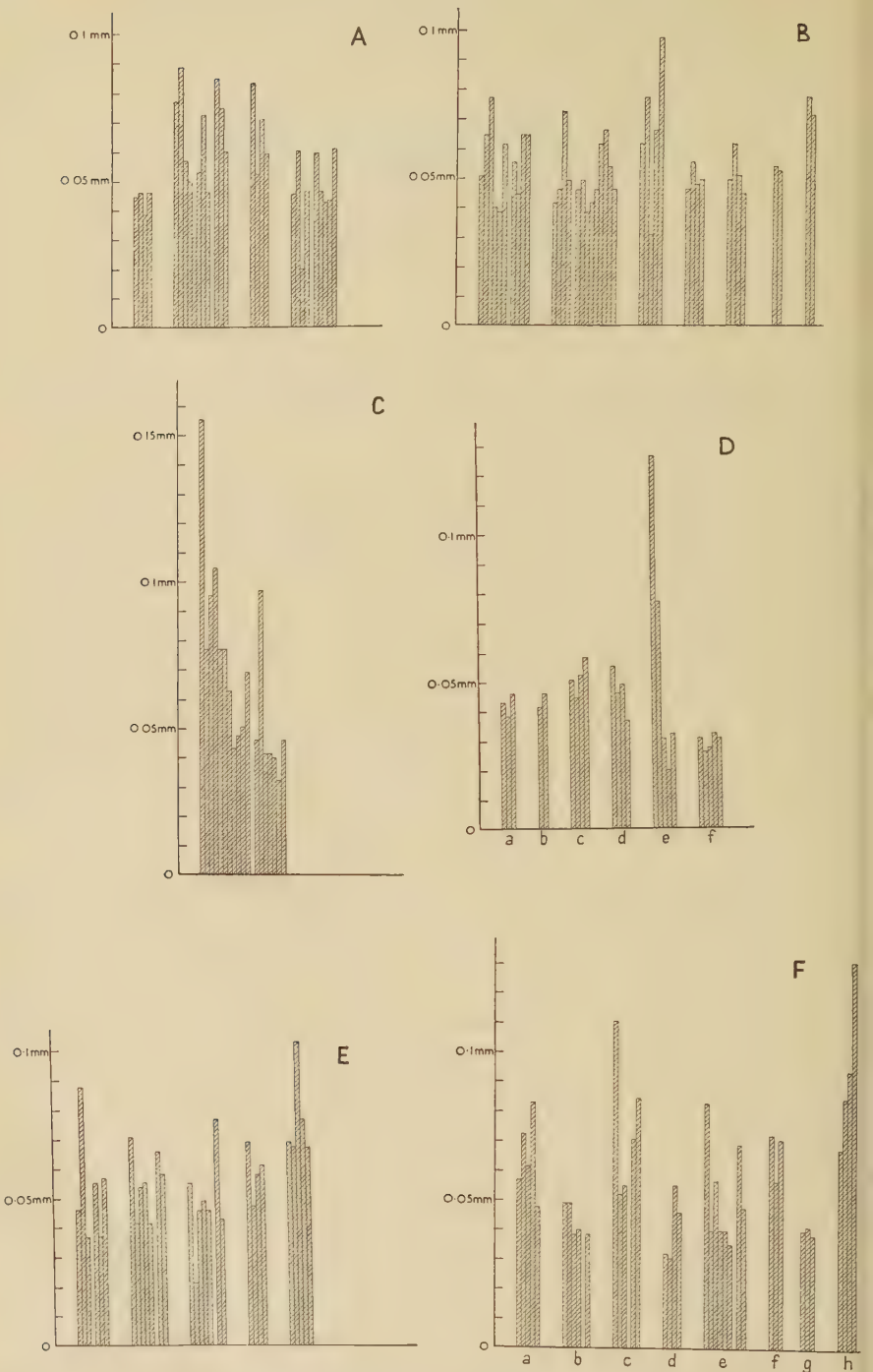

FIG. 1. Diagrams showing the lengths of myelin segments in sub-epidermal plexuses. A–E minnows; F, a gudgeon. For explanation see text.

arge differences between the lengths of these internodes and those reported elsewhere.) The mean of the two sets of twenty-eight measurements was 55·6 μ and the standard deviation of the internode length 16·4 μ. These internodes are those shown in fig. 1, A; measurements made on other minnows are recorded in fig. 1, B, C, D, and E. All the measurements recorded in any one diagram are from the same animal; each vertical column of the histogram represents the length of a single myelin segment, and successive internodes on a single fibre are shown in blocks, the proximal end to the left, the peripheral to the right. Blocks separated by only the width of a single column from the preceding block represent branches of the same fibre, though the point at which the fibre branched is not indicated. The blocks separated by more than the width of a single column each represent a separate fibre from the same preparation. The diameters of the measured fibres ranged from something over 1 μ ('fine' fibres) to about 2 μ ('coarse' fibres); exact measurement was not possible. All the fibres measured in the fish preparations were of the sub-epidermal plexus.

The length of the shortest internodes seen was 20 μ. These were rare on the thicker cutaneous fibres (see fig. 1, A, E) but were more common on the finer fibres; the average internode length on the 'fine' group of fibres was lower than on those of greater diameter, as may be seen by comparing blocks *c*, *d*, *e*, and *f* in fig. 1, D with fig. 1, A, B, C, and E. In fig. 1, D, blocks *a* and *b* represent the terminal internodes of 'coarse' fibres closely adjacent to the 'fine' measured fibres (blocks *c* to *f*); fibre *e*, which is fine, contains two long internodes, which may have arisen by the coalescence of myelin segments across a node (see Thomas and Young, 1949; or Speidel, 1933). There is in any case a wide variation in internode length. The presence of scales may sometimes cause irregularities as the advancing edge of a growing scale will stretch that part of an adjacent nerve fibre which passes round the scale towards the surface; in fig. 1, F, which represents fibres from a gudgeon, *Gobio fluviatilis*, block *h* represents such a fibre, the myelin segment at the edge of the scale being considerably longer than the proximal internodes which lie under the scale.

It was apparent that in the sub-dermal plexus the internodes were usually longer than in the sub-epidermal plexus, that is, that internode length decreased as the fibre neared its end; this was confirmed in osmium tetroxide preparations, where it was seen that diameter also decreased towards the periphery. Measurements were not made as it was difficult to identify a single fibre for any distance owing to the crossing of the numerous fibres in these nerve bundles, which are larger than those of the sub-epidermal plexus. In the frog, *Rana temporaria*, on the other hand, individual fibres may be traced in larger nerve bundles; accordingly, preparations of frog skin were made, by subcutaneous injection of methylene blue, as in the fish. Internode length was measured on fibres approaching and ramifying in the skin of the thigh; representative results are shown in fig. 2, A, B, and C. It will be seen that here, as in the fish, the peripheral internodes are often less than 150 μ in length.

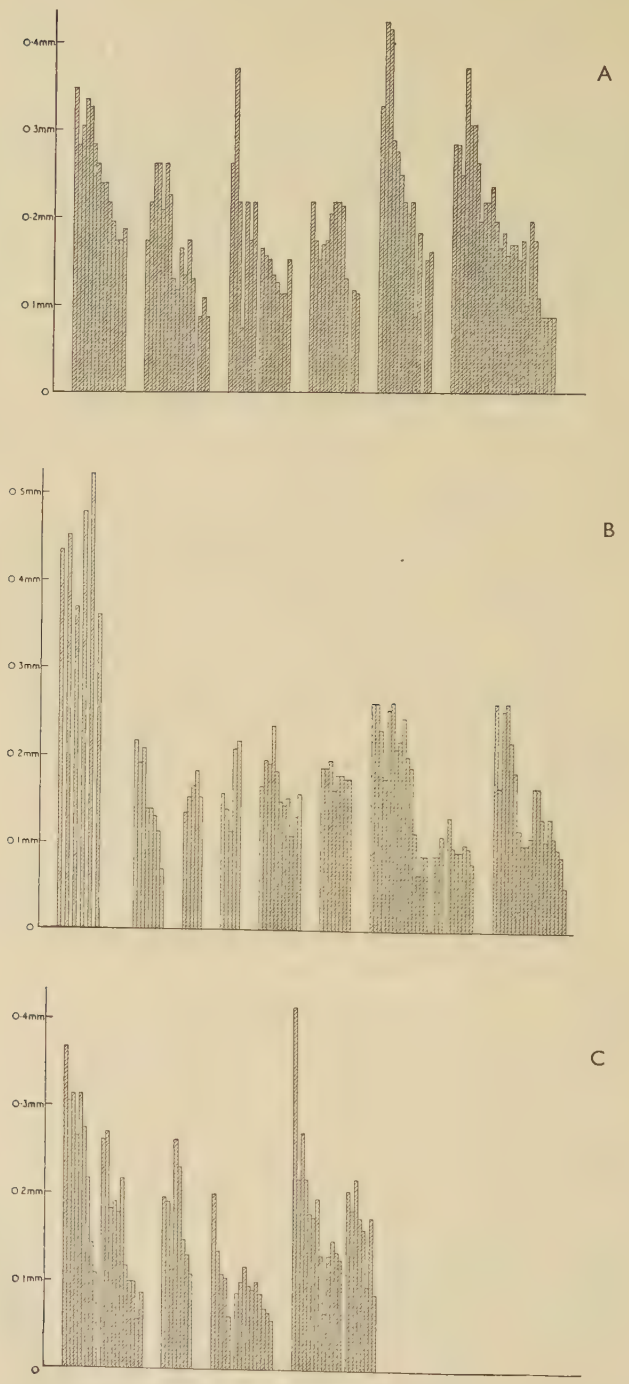

FIG. 2. Diagrams showing the lengths of internodes on fibres entering the dermal plexus of the thigh of frogs. A and B, length of thigh 2·5 cm.; C, length of thigh 1·5 cm.

The various fibres represented cannot be directly compared as only that part of a fibre which was well stained could be studied and the distance of the well-stained segments from the final node is not constant. Though there is considerable variation in internode length along any part of the fibre, it is clear that, on the whole, there is a progressive decrease in the length as the fibre approaches its termination (that is, towards the right of each block in the diagrams); this is correlated with an increased frequency of branching, a fact which does not appear in the figures. The columns at the extreme left of fig. 2, B represent individual internodes from a larger nerve bundle, approaching the skin; here the nodes are farther apart than in the dermis. The statement of Takahashi (1908), that the average length of the internodes on the fibres in the nerves of the leg diminishes towards the periphery, does not refer to the same phenomenon, for his measurements were made at different levels of the nerve trunks in the leg, not in the finer cutaneous branches.

No measurements were made in the larger nerve trunks of the leg. Boycott (1904) measured the internodal lengths found in the sciatic nerves of frogs of various sizes; his figures are quoted and further analysed by Hatai (1910). It is not possible to make direct comparisons of the fibres measured by Boycott and those seen in the skin plexus, unless a detailed analysis of the range of fibre diameters be made. The diameter decreases as the fibre branches, though the various branches are not always of equal calibre. Furthermore, though the coarsest fibres stained were selected for measurement, these were not necessarily the largest fibres present. Only the minimum length of internode is relevant in the present instance; Boycott records none of less than 200 μ in the sciatic nerve. In the dermal plexus of the frog no internodes less than 50 μ in length were seen, but myelin segments of less than 100 μ were common in the branches; these were not necessarily at the extreme end of the medullated part of the fibre, for measurements could not be made beyond the point at which the fibre turned towards the surface.

Young (1944) and Thomas and Young (1949) suggest that a limiting physical condition, such as the stable length of a droplet under surface tension, may be one of the factors determining the minimum length of an internode. Hiscoe (1947) disagrees on the grounds that the myelin is not continuous from one node to the next but is interrupted by the incisures of Schmidt-Lantermann; she also considers that the laminated microstructure of myelin (citing Schmitt and Bear, 1937) precludes the consideration of any simple forces of surface tension. Whether the minimum internodal length of the nerve trunks is taken as 200 μ or as 150 μ, it is evident that the myelin segments in the dermis may be shorter than this. If therefore it is suggested that the minimum length of the myelin segment does depend on surface tension, or on some similar physical factor, it must be shown that the conditions affecting this factor differ in the two environments, to such an extent as to account for the difference in minimum length. If the myelin segment is regarded not as a stable droplet but as a droplet held in dynamic equilibrium by several factors which include the passive (or elastic) limiting pressure of

the neurilemma and the surrounding tissues, its form or length might perhaps be the more readily modified by external factors.

The decrease in the lengths of the internodes as the fibre approaches its end may be partly explained by the fact that the proximal parts of the fibre were medullated first; during growth these proximal segments must have been stretched more than those which were established later, but the decrease in internode length as the fibres pass through the dermis is sudden enough to make it unlikely that such a factor alone is responsible. The fact that growth has taken place may mean that the minimum length in the nerve trunks was in fact less than 150 μ when the myelin first appeared; Young (1945) states that in the foetal rabbit the myelin segments on their first appearance were 150 μ in length, and that earlier, when the Schwann cells (nuclei?) were fairly regularly spaced at intervals of 100 μ or less, no myelin was detectable.

It has also been suggested that the length of the young Schwann cell itself may govern the position of the nodes (Young, 1945; Hiscoe, 1947). If this were so, the difference of minimum internode length in the nerve trunks and in the skin might reflect a difference in the initial periodicity of the Schwann cells, perhaps due to such environmental factors as a restriction in the length of the Schwann cells where two or three come to occupy the limited distance between two points of branching of a fibre; in such a case the myelin segments being produced under their respective influences would also be short. In regenerated nerves of adult rabbits the internodes are all relatively short, ranging from 150 μ to 700 μ (Vizoso and Young, 1948); although Vizoso and Young speak of 150 μ as the lower limit of internodal length in these regenerated nerves, they record a few myelin segments only 100 μ long on the finer fibres. In the minnow, where the shortest internodes seen measured 20 μ, the length of a Schwann cell nucleus was 10 μ; in photographs of regenerating rabbit nerve published by Holmes and Young (1942) most of the Schwann cell nuclei are longer, about 20 μ in length.

It can be concluded that the minimum internodal length is less than that previously reported and that, even when allowance has been made for subsequent growth, the internodes in the dermis may be considerably shorter than those in the nerve trunks.

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Receptor Elements in the Thoracic Muscles of *Homarus vulgaris* and *Palinurus vulgaris*

By J. S. ALEXANDROWICZ

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With three plates (figs. 4, 10, and 13)

Summary

1. In the thoracic muscles of *Homarus vulgaris* and *Palinurus vulgaris* the presence receptor elements of various kinds has been recorded. Muscle receptor organs longing to the same category as described previously in the abdomen have been und in the two posterior (7th and 8th) thoracic segments. According to their situan one lateral and one median receptor may be distinguished on each side of each gment. Like those in the six abdominal segments they are linked with the system the extensor muscles. The topography of these organs in relation to that of the oracico-abdominal muscles is described. There are certain differences in their locan between the two species.

2. Receptor organs of this category, with one exception, are composed of a special n muscle and a nerve-cell ending in connective tissue intercalated in this muscle. ie lateral receptor of the 7th thoracic segment in *Homarus* has no muscle of its own d terminates on connective tissue fibres accompanying an ordinary muscle. In the rve supply of these receptors in the thorax the same elements as in the abdomen, . the motor nerves and the two accessory nerves, can be distinguished, but their stribution in the thorax has certain special features.

3. It is assumed, in view of certain differences in the appearance of the nerve-cells d their processes, that the muscle receptor organs of this category are of two types each of the segments.

4. Nerve-cells regarded as receptors of a different category have been found in me of the muscles inserting in the median surface of the epimeral plate. These lls, termed 'N-cells', are smaller elements than those of the first category and have t a special muscle of their own, but end with long processes between the fibres of e ordinary muscles. In each of the species investigated five such elements have been und. No evidence is as yet available as to whether they are present exclusively in e thoracic region or are more generally distributed.

5. It is suggested that the N-cells may represent more primitive forms of muscle ceptors, and that the receptor organs of the extensor muscles in the thorax and domen are more highly evolved forms.

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Contents

Introduction

THE present communication continues the description of muscle re ceptor elements in crustaceans, the first part of which dealt with thes organs in the abdomen of *Homarus vulgaris* and *Palinurus vulgaris* (Alexan drowicz, 1951).

The methods used were the same as those previously described.

Observations

Homarus vulgaris

Topography of the dorsal thoracic muscles

To locate the muscle receptors it is essential to have a good knowledge o the arrangement of the muscles of the thorax as they appear when dissected so as to expose the receptors. On the left side of fig. 1 the thoracico-abdomina muscles are drawn as they come to view, after removal of pieces of the cara pace and the interarticular membrane and after cutting through a thi membrane-like muscle (here called m. membranaceus) which inserts int this membrane. The large muscle masses flanking the heart have been de scribed by Milne Edwards (1834), Schmidt (1915), and Daniel (1930), bu

Unfortunately there is no uniformity in the nomenclature used by these writers and the same name has even been applied to different muscles. Without further discussion of this I shall use the term 'dorsal thoracico-abdominal muscles' for those attaching at the dorsal anterior margin of the 1st abdominal segment, and 'lateral thoracico-abdominal muscles' for those whose posterior attachment is situated on the lateral side of the interarticular membrane.

The mass of the dorsal thoracico-abdominal muscles is composed of three muscle units for which the terms first, second, and third dorsal thoracico-abdominal muscle are proposed. They all have their posterior attachments at the front edge of the 1st abdominal segment, the first muscle partly overlying the second and the latter partly overlying the third. Their fibres run in an antero-lateral direction, but at a different angle in each of the three muscles. Moreover, on fresh material a certain difference in colour is noticeable between the first and second muscles on the one hand, and the third on the other, the two former being somewhat yellowish; histological examination shows that the first and second muscles are composed of thicker and more sparsely cross-striated fibrils than the third.

In their antero-lateral course the fibres of all three muscles curve in a central direction, so that their anterior attachments, which are on the epimeral plate, are not visible from the dorsal side even when one head of the lateral thoracico-abdominal muscle is cut through as shown on the right side of fig. 1. In order to obtain preparations in which the muscles can be examined in their whole length, they have to be cut out with the adjoining chitinous parts and stretched, as shown in fig. 2, C, with the median surface of the epimeral plate turned upwards in the manner indicated in diagrams A and B in the same figure. The anterior attachments of the three dorsal muscles are nearly in line, but their independence is quite distinct; it may be noted that the third muscle is composed of three portions whose insertions in the epimeral plate are separated. It should also be mentioned that the dorsal muscles are covered by a comparatively strong fascia with fibres predominantly transverse in direction.

The lateral thoracico-abdominal muscle consists of two large heads and two feeble slips of muscles, all having their posterior insertions in the chitinous plate which projects from one of the calcified reinforcements of the interarticular membrane between the abdomen and the thorax and passes into the muscle. The first head, the only one which can be seen in fig. 1 on the left side, is attached anteriorly to the carapace at its cervical groove. The second head can be seen after the first one is cut through and hooked up as shown on the right side of the same figure. The whole of this second head is better seen in fig. 2 as well as the two tiny bundles mentioned above. Since one of the latter has an important place in the following account the term 'accessory muscles' will be used for short.

Other muscles which are represented in fig. 2 and which will be considered later are: m. contractor epimeralis, a flat muscle which, curiously enough, has

both its attachments on the epimeral plate, and m. attractor epimeralis (
which only a part appears in the figure), a series of short bands of muscl
stretching between the carapace and the anterior and dorsal edges of th

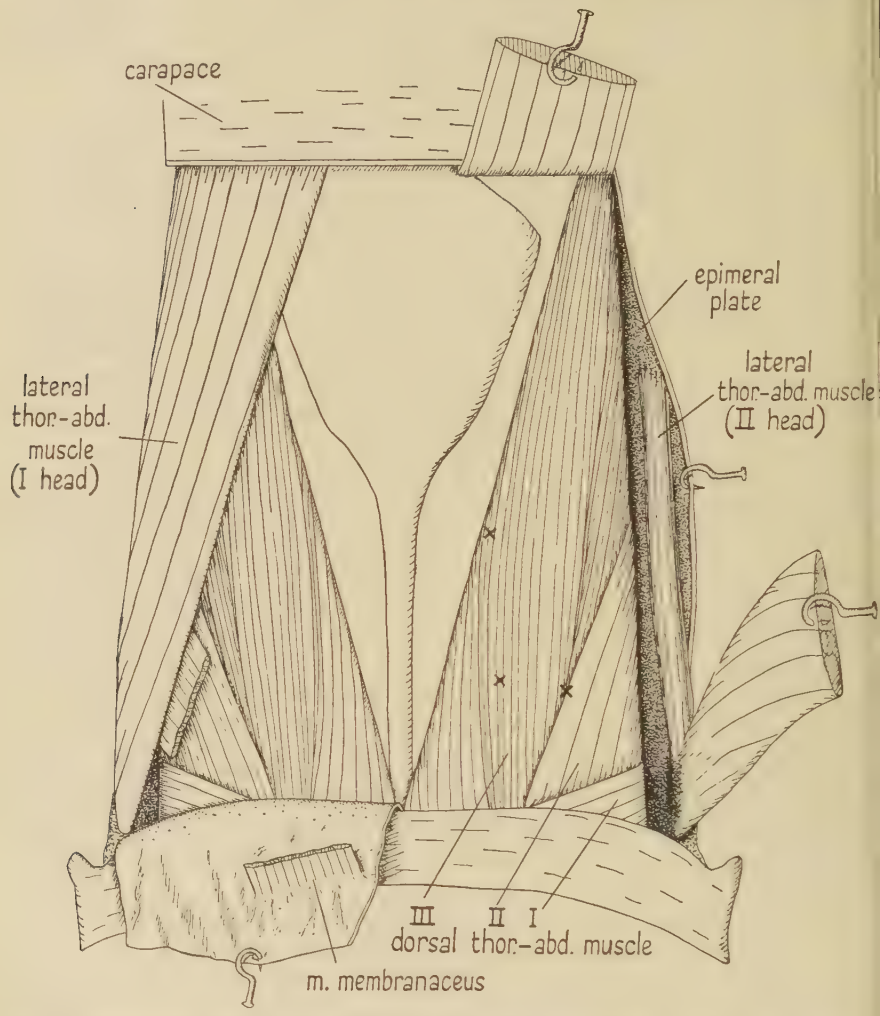
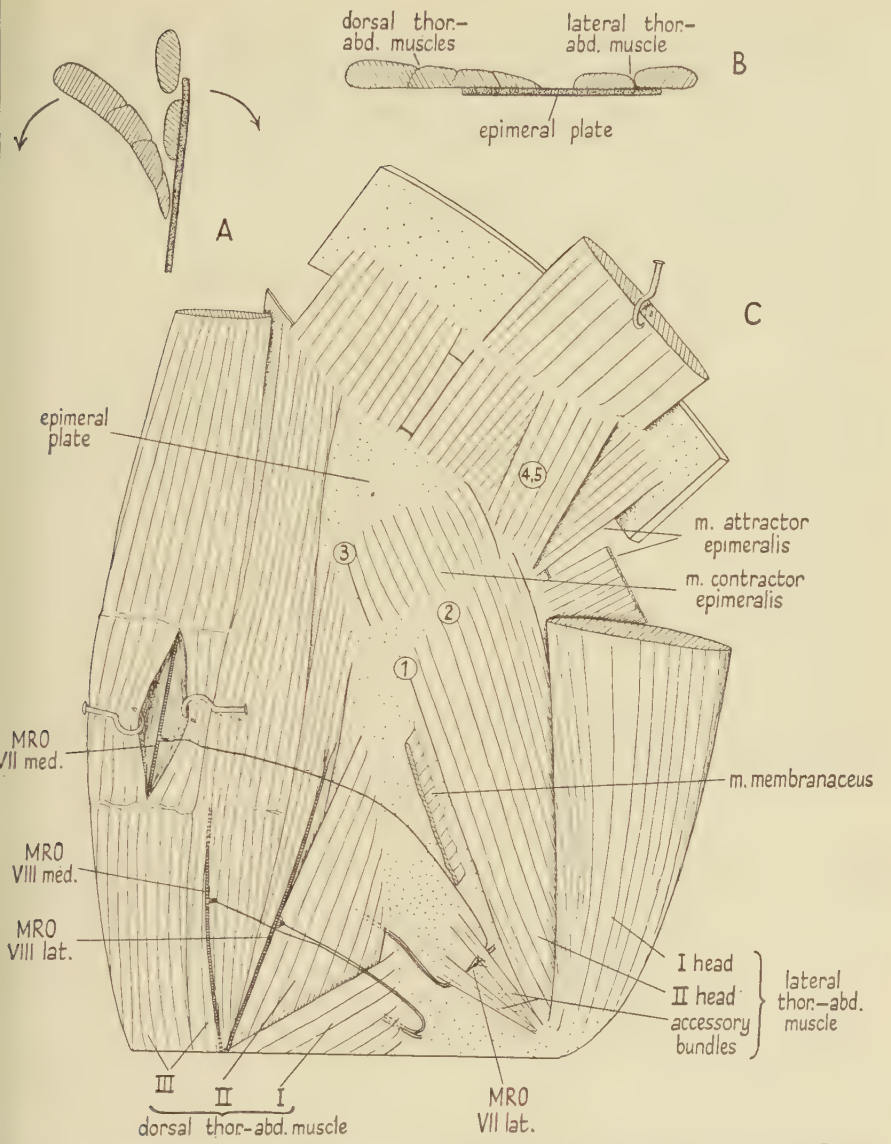


FIG. 1. *Homarus vulgaris*. Topography of the thoracico-abdominal muscles seen from th
dorsal side. In the left bottom corner the interarticular membrane attaching at the 1st abdom
nal segment is pulled back, showing its ventral side with the insertion of the m. membranaceu
which has been cut through. On the right side the 1st head of the lateral thoracico-abdomin
muscle is sectioned. The crosses indicate the position of the cells of the muscle receptor organ

epimeral plate. The terms for the last two muscles have been adopted b
Schmidt (1915) in his monograph of the muscle system of *Astacus fluviatili*
It may be observed that the arrangement of all the muscles enumerated abov
in *Homarus* is much the same as in *Astacus*, a fact which was not unknown t
Schmidt.



IG. 2. *Homarus vulgaris*. A, B. Diagrammatic transverse sections of the dorsal part of the horax of the right side showing the way in which the epimeral plate and the thoracico-bdominal muscles have to be displaced in order to obtain a preparation as in C with the orsal and lateral thoracico-abdominal muscles brought into one plane. C. View of the thoracico-bdominal muscles with their attachments to the epimeral plate and the topography of the eceptor elements. The inner portion of the third dorsal thoracico-abdominal muscle inserting nore anteriorly is sectioned; the first head of the lateral thoracico-abdominal muscle is cut nrough and its anterior portion inserting into the carapace is hooked upwards. MRO lat. ned., lateral and median muscle receptor organs of the 7th and 8th segments. The encircled numbers indicate the positions of the five N-cells.

Receptor elements

The receptor elements which are connected with the thoracic muscles a of various categories and the problem of their classification will be discusse later. For purposes of description they may be sorted into three groups: (muscle receptor organs in the thoracico-abdominal muscles, (2) receptor el ment of the anterior accessory muscle, and (3) receptor elements of oth muscles inserting in the epimeral plate.

Muscle receptor organs in the dorsal thoracico-abdominal muscles

Topography of the receptor organs

Within the area of the dorsal thoracico-abdominal muscles three recepto organs are present similar in character to those in abdominal segments, i. consisting of a nerve-cell connected with its own special muscle unit. Judgin from the nerves with which the axons of the cells are associated it appea that two of them belong to the 8th and one to the 7th thoracic segments.

The MRO (muscle receptor organs) of the 8th segment may be easil located, for their cells are situated quite superficially and stain infallibly wit methylene blue. They are always at a certain distance from each other an accordingly, the two MRO may be distinguished as lateral and media respectively.

The MRO of the 7th segment, which, as will be explained later, shoul be regarded as the median receptor elements of this segment, lies more deepl among the bundles of the inner portion of the third muscle, and therefor certain difficulties have to be overcome in order to locate it.

The positions of the cells of the three receptor organs in the dorsal muscle *in situ* are indicated by crosses in fig. 1. The muscle components of thes MRO are situated as follows (fig. 2). The muscle belonging to the lateral ce of the 8th segment (MRO VIII lat.) originates at the anterior margin of the 1s abdominal segment close to the insertions of the inner fibres of the secon dorsal thoracico-abdominal muscle. It may run forward at a slightly les oblique angle from the fibres of this muscle and thus come to lie on the thir dorsal muscle, but always near the edge of the second. In its anterior cours it is flattened and closely applied to the fascia covering the dorsal muscle to which it becomes attached, dividing sometimes into several slightly diverg ing slips. The connective tissue fibres accompanying the muscle pass ove into the fascia and thus strengthen its attachment.

The muscle of the median MRO of the 8th segment (MRO VIII med originates at the edge of the 1st abdominal segment near to the muscle of th lateral one but at a slightly deeper level. Running on the fibres of the middl portion of the third dorsal thoracico-abdominal muscle it forms an acut angle with the muscle of the lateral MRO. In the greater part of its course remains above the dorsal muscle, but more anteriorly it comes to lie at th same level as its superficial fibres and ends in this position, inserting into th interstitial connective tissue.

The muscle of the median MRO of the 7th segment (MRO VII med.) lies ıong the bundles of the inner portion of the third dorsal thoracico-abdominal ıscle. In the middle part of its course it is situated not far from the surface, t anteriorly and posteriorly it penetrates gradually deeper and is very diffi-lt to follow in dissections. Its attachments are on the connective tissue tersections of the dorsal muscle.

It should be emphasized that all three muscles, like those in the abdominal gments, must be considered as independent muscle units; this is especially teresting in the case of the receptor muscle of the 7th segment which though rrounded by the bundles of the ordinary muscles retains its individuality g. 5, A).

ructure of the muscles

The muscles of all three receptors are similar in structure to those in the dominal segments, being composed of bundles of myofibrils ensheathed by rying amounts of connective tissue; the latter is more abundant in the teral receptor of the 8th segment. It seems very probable that the individual ndles of myofibrils, apart from their interruption by the intercalated tendon, not run the whole distance between the two ends of the muscle but become tached to the surrounding connective tissue. The varying diameter of the uscles, ranging from about 100 to 200 μ, as observed in whole mounts, may partly due to this behaviour of their elements, but also to a large extent sults from the shape of the muscle which varies in cross-sections from lindrical to flat in different places. It is difficult to ascertain which of these riations are natural and which are produced artificially. Transverse sections ake it clear, however, that the muscle of the lateral MRO of the 8th seg-ent is the stoutest of the three.

There are differences in the histological structure of the muscles. The ickest myofibrils with coarse cross-striation are in the lateral MRO of the 8th gment, the thinnest with the finest striation in the median one of the same gment; those of the 7th segment are intermediate in appearance.

As in the receptors of the abdomen the muscle tissue of these organs is placed in a certain region by connective tissue fibres and this is also the ea of expansion of the terminations of the cell dendrites. However, only in e median MRO of the 8th segment is the whole of its muscle interrupted such intercalated tendinous tissue; in the lateral MRO of the same seg-ent this tissue replaces only about two-thirds of the myofibrils, whereas the st of them run through this area without being interrupted; in the median RO of the 7th segment the intercalated tissue occupies an even smaller gion, both in length and width, looking like a patch in the muscle bundle.

erve-cells

The nerve cells of the three MRO show the same characteristics as in the dominal segments, viz. they have several shorter dendritic processes ending the muscle of the receptor organ and a long one, the axon, running towards

the central nervous system; each cell has, however, particular features of i own.

The lateral cell of the 8th segment has comparatively long dendrites sprin ing as a rule from various points of the cell-body (figs. 3, A, C, E, and 4, B). N

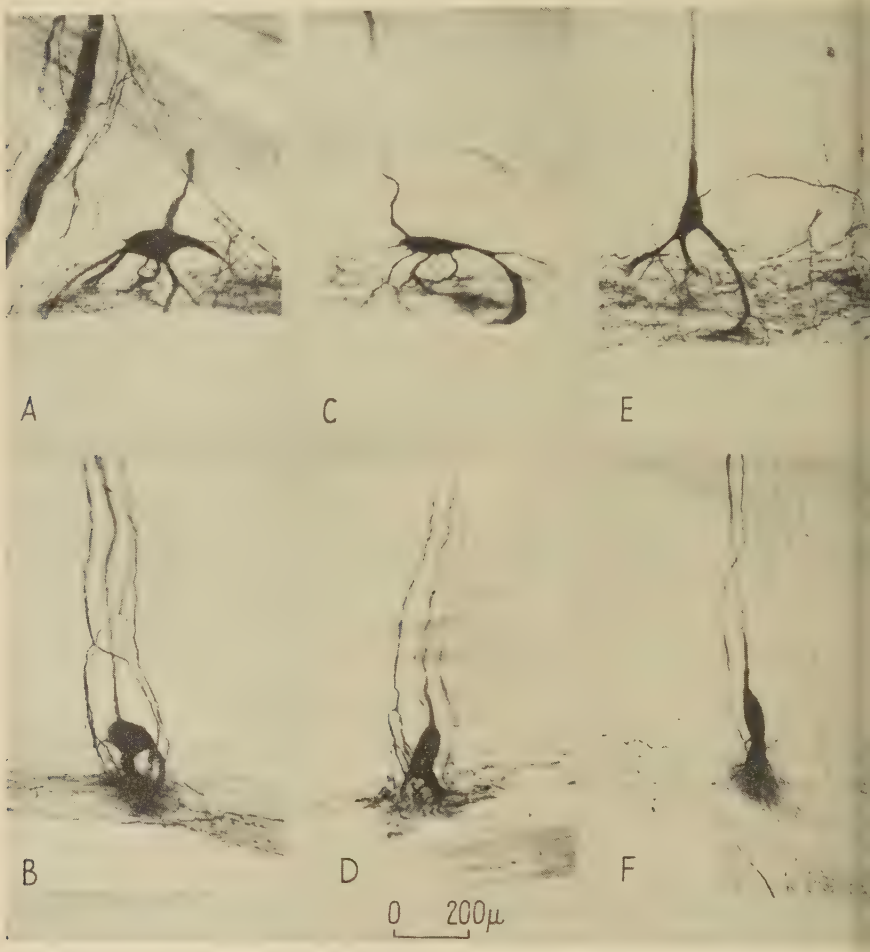


FIG. 3. *Homarus vulgaris*. Photomicrographs of the nerve-cells of the muscle receptor orga of the 8th thoracic segment showing the difference between the lateral cells (upper row) a the median ones (lower row) and the variations in their shapes. The pairs A, B, and C, D, ha each been taken from the same specimen. *Note*: in A and C, dendrites curving round t muscle; in E, one of the dendrites projecting sideways (cf. fig. 4, B); in B the two accesso nerves running alongside the axon and the motor fibre crossing the muscle obliquely. D and F only the thick accessory nerve is well stained.

uncommonly one of them, longer than the others, gives the appearance being a different kind (fig. 3, E); it can, however, be shown that it only tur round the axon of the median receptor cell and terminates near the oth processes (fig. 4, B). Its elongation is obviously due to the changing of positio

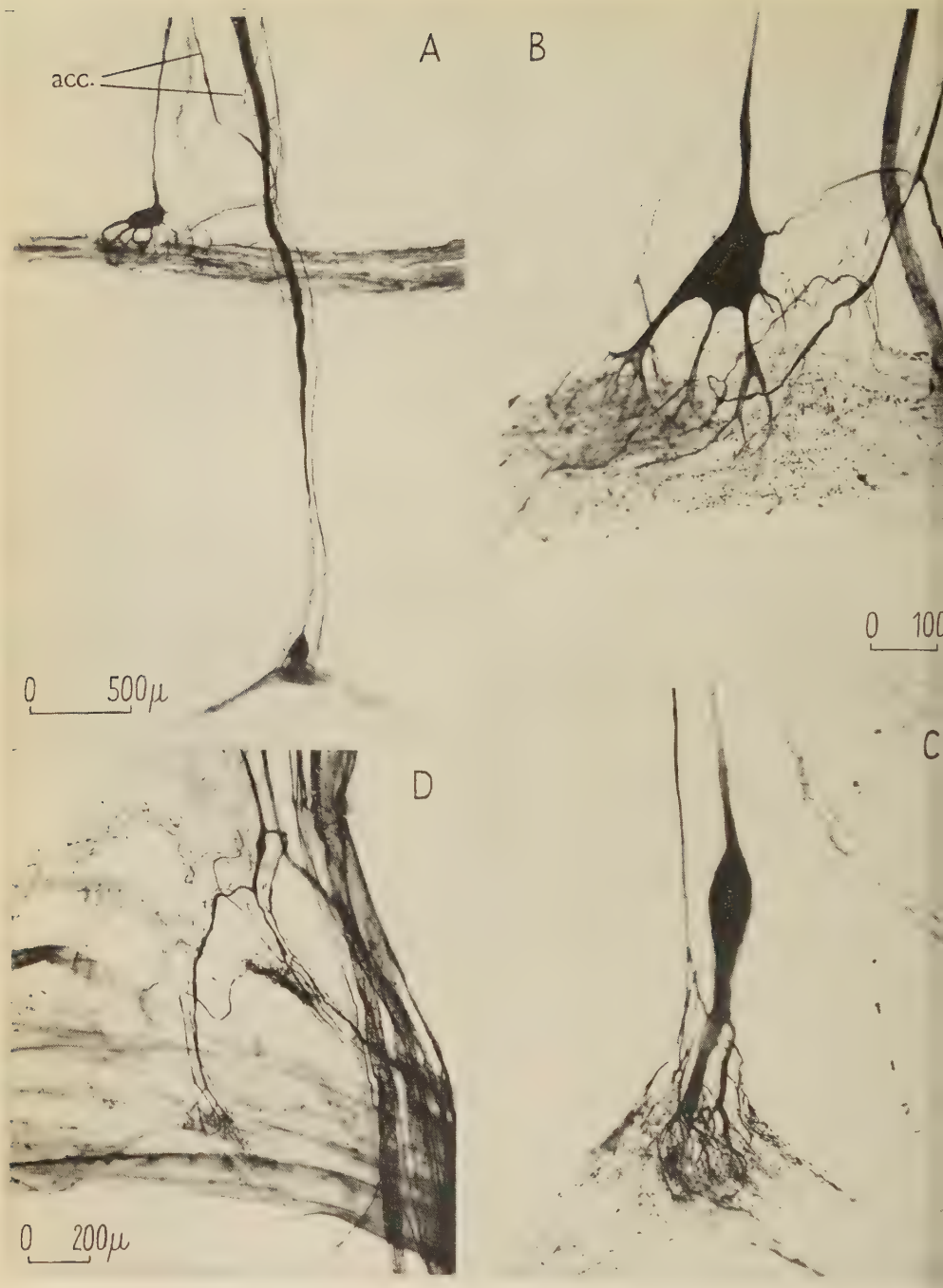


FIG. 4

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f the two elements during the growth of the animal. The dendrites approach ıe receptor muscle at various points on its circumference, even on the oppo- te side to that on which the cell is situated, when one or more of them curve round the muscle before penetrating into it (fig. 3, A, C). The variable number f dendrites which may sometimes spring from the axon, and their often ssymetric arrangement, give this cell a remarkable multiformity in appearance igs. 3, A, C, E, and 6).

The endings of processes are distributed in the intercalated connective ssue. As a fair number of the myofibril bundles pass through this region it difficult to ascertain whether or not they receive some of the nerve ter- inations also, though it seems more probable that all the ramifications end ı the connective tissue.

The median cell of the 8th segment is less variable in shape. Its dendrites rise as a rule from the distal half of the cell-body and often begin with two r three stouter roots. Their abundant ramifications end on a more restricted rea than do those of the lateral cell; even when some of the dendrites happen) arise nearer to the axon they do not diverge but run close to each other, and ıis arrangement of the processes into a more thickset tuft may be considered s a characteristic feature of the median cell. Although both lateral and median ells may exhibit variations in shape and type of branching tending towards ach other in appearance, there is usually no difficulty in recognizing one rom the other at first sight (cf. cells in the lower row of fig. 3 with those in ıe upper row). The differences in the types of branching of the two cells are lustrated in fig. 4, B, C.

The cell of the MRO of the 7th segment resembles the median cell of the th, but it is a little smaller and its tuft is also smaller. It shows, besides, a reater variability in its shape: some of the unusual forms observed may be ue to the situation of the cell amongst the muscle bundles; others might asily have been produced artificially during dissection to find this hidden IRO. The processes may arise from a single common root or in a number irectly from the cell (fig. 5, A, B).

FIG. 4 (plate). Elements of the muscle receptor organs in *Homarus vulgaris*. Photomicrographs made from preparations stained with methylene blue, fixed with ammonium molybdate, and mounted in xylene dammar.

A. Receptor cells of the 8th thoracic segment of the right side with portions of their muscles. The much larger apparent size of the lateral cell is due to the staining of its capsule. The fibres running alongside the axon of the median cell are the branches of the two accessory nerves *acc*.

B. Lateral nerve-cell of the 8th segment with its dendrites penetrating the receptor muscle; one of the dendrites projecting sideways turns round the axon of the median cell. The branches of the thick accessory nerve approach the dendrites from the right and those of the thin one from the left side.

C. Median receptor cell of the 8th segment showing a pattern of branching differing from that of the lateral cell as seen in B. The fibre running down the axon is the thick accessory nerve.

D. Lateral receptor cell of the 7th segment with a portion of the accessory muscle and of the nerve trunk. Only two of the cell processes are well stained up to their endings; one of them, in the middle of the figure, is accompanied by branches of the two accessory nerves.

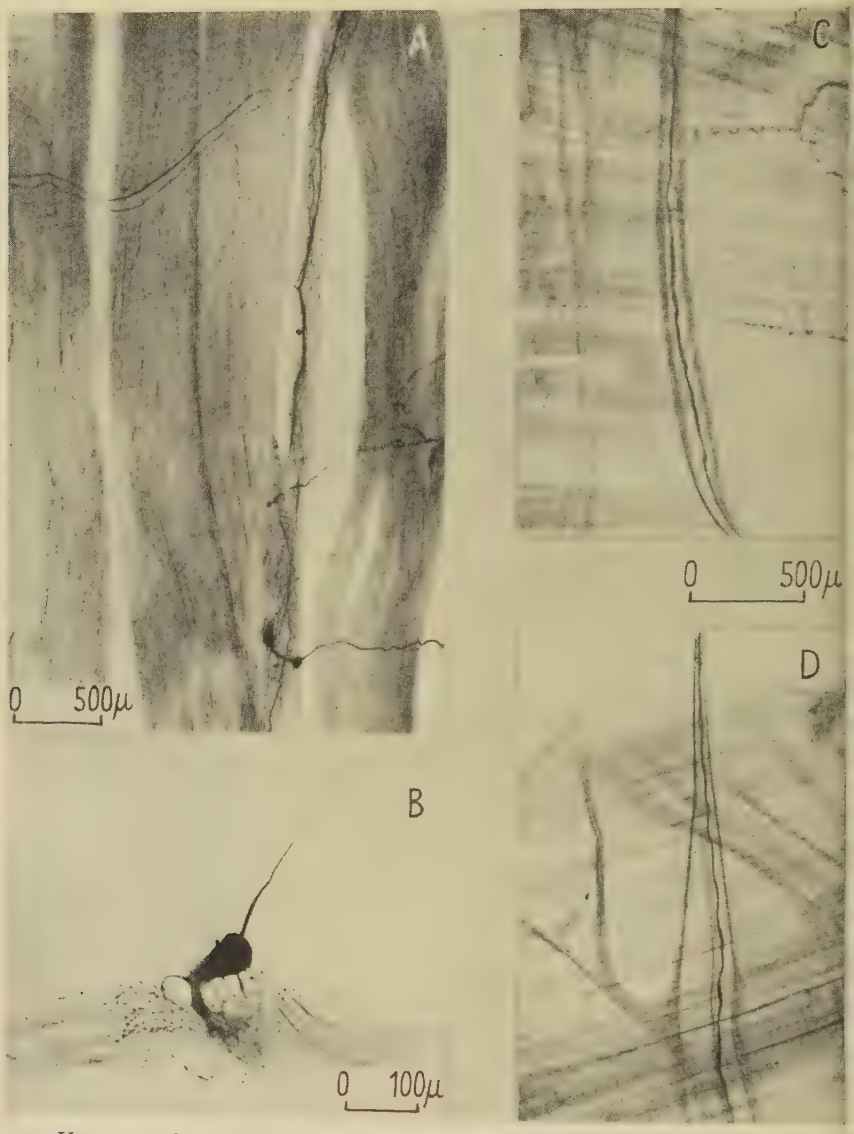

FIG. 5. *Homarus vulgaris*. Photomicrographs showing the elements of the muscle recept organ of the 7th segment. A. Receptor muscle amongst the bundles of the third dors thoracico-abdominal muscle; note its innervation and the nerve-cell with a long distal pr cess; B. Nerve-cell with several dendrites arising from the cell-body; C, D. Axon of the nerv cell in its course on the surface of the dorsal muscles accompanied by connective tissue fibre

All three nerve-cells show the same histological structure as those in th abdomen. Like them they are also enclosed in a capsule which in some pr parations is clearly visible (fig. 6, A). The connective tissue surrounds th capsules with several concentric layers; it is only occasionally noticeable i methylene-blue preparations, but in sections it may be very distinctly seen.

The axons of the cells of the 8th segment undergo remarkable changes in eir calibre. After rising from the cells they taper for some distance but after short course their diameters increase and reach conspicuous dimensions. his character of the axon of the median cell is well illustrated by the photo- icrograph (fig. 4, A); the stoutest fibre in fig. 4, B and in fig. 6 is the axon of ie median cell as well. The axon of the lateral cell, of which only a portion seen in these figures, grows distally to about the same thickness as that of ie median cell. These unusually large dimensions must be partly due to attening of the cylindrical fibres, but they can be regularly observed in fresh ssues before fixation.

The axon of the median cell always crosses the lateral MRO behind its ell and usually at a certain distance from it. Passing outwards they come to e near to each other and, running transversely on the surface of the second nd first dorsal thoracico-abdominal muscle, curve ventrally to join the nerve f the 8th thoracic segment (fig. 2).

The axon of the median cell of the 7th segment is at first directed dorsally, ut after emerging on the surface of the dorsal muscles turns laterally. As it pproaches the epimeral plate it crosses the second dorsal thoracico-abdo- inal muscle near to the apex of the triangle formed by the inner fibres of is muscle and its line of insertion; passing on to the median surface of the pimeral plate it runs towards the nerve which crosses the two accessory uscles on their lateral side, i.e. between these muscles and the epimeral late (fig. 2).

This axon is thinner than those of the cells in the 8th segment but even so is thicker than the nerve fibres in its neighbourhood, and by this feature nd the absence of branching it can be identified in preparations while stain- g. This is important, for this nerve fibre has to serve as a guide leading to ie place where the hidden cell may be found and exposed to view. The ngth of the axon from the cell to where it joins the nerve of the 7th segment, easured in such preparations as shown in fig. 2, amounts to 2 cm. in medium- zed specimens.

All three axons in their courses between the surface of the muscles and the scia covering the latter are flanked by connective tissue fibres. A special rotection is given to the axon of the cell of the 7th segment; in its long assage across the dorsal muscles it is accompanied by a few fibres which do ot surround it as a sheath but run alongside it, and are at some points rengthened by deviating branches passing over into the fascia (fig. 5, C, D). imilar accompanying fibres secure the axon in its position at the point at hich it passes on to the epimeral plate and also on the plate itself.

Nerves

It may be recalled that in the abdomen in the same species three sorts of erves entering into relation with the MRO have been distinguished: (*a*) otor nerves, (*b*) thick accessory nerve, and (*c*) thin accessory nerve. In all ie abdominal segments the disposition of these nerves is about the same; in

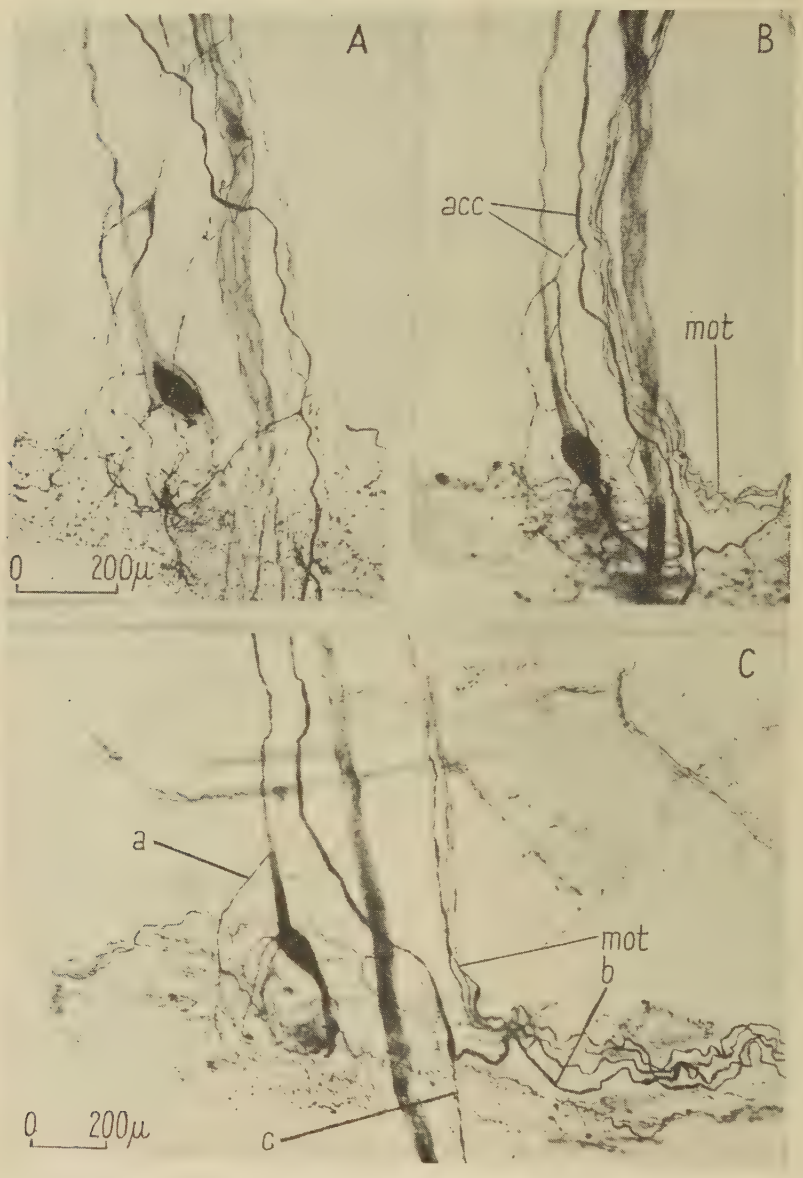

FIG. 6. *Homarus vulgaris*. Photomicrographs of the lateral muscle receptor organ of the 8th segment of the right side showing: in A nerve-cell with a distinct capsule; in B, all three kinds of nerve fibres, i.e. the motor fibres *mot*, and the two accessory nerves *acc*, the thin one approaching the cell from the side of the axon; in C motor fibres *mot*, and the branches of the thick accessory nerve to the cell dendrites (*a*), to the muscle (*b*), and to the median receptor cell (*c*). The stoutest fibre in all the three figures is the axon of the median cell.

the thorax, however, the innervation of each of the MRO has its particular features.

Lateral MRO of the 8th segment. The motor fibres of this MRO arise from various nerves supplying the neighbouring muscles. Some of them spring from the nerve of the first dorsal muscle and run alongside the axons. They correspond to those in the abdomen which have been termed 'main motor fibres'; in the thorax, however, they are of comparatively small calibre. Other motor elements corresponding to 'additional motor fibres' in the abdomen are given off by the nerves supplying the muscles on which the receptor muscle is lying and approach this muscle at points more or less distant from the cell. One of them, more easily seen, passes on to the muscle near to its posterior end.

The thick accessory nerve is the stoutest of all the fibres supplying this MRO. It runs as a rule close to the axons and on nearing the lateral MRO divides into several branches (fig. 6, C): some of them (*a*) are short and distribute their ramifications in the same area where the cell dendrites end, others, the longer ones (*b*), pass on to the receptor muscle and supply it in its whole length; finally, one (*c*) continues the course of the nerve from which they all arise and runs towards the median MRO.

The thin accessory nerve follows the same route as the thick one but seems to be less closely associated with the latter, joining rather the bundle of the main motor fibres (fig. 6, B). It gives off branches to the area of distribution of the cell dendrites and one branch running towards the median MRO. The fibres of the two accessory nerves approaching the lateral nerve-cell may be seen in fig. 4, B and those running to the median cell in fig. 4, A.

Median MRO of the 8th segment. The median MRO receives its motor innervation from the fibres supplying the third dorsal muscle and, as the nerves to the latter travel under the second dorsal muscle, the branches to the MRO appear to emerge from underlying muscle bundles. No motor fibres are present corresponding to the main motor fibres of all MRO hitherto described, i.e. running down to the receptor muscle alongside the axon of the nerve-cell, and all the fibres seen in the pictures accompanying this axon (fig. 3, B, D, F; fig. 4, A, C) are elements of the accessory nerves. The latter distribute their endings in the intercalated tendon of the muscle, i.e. in the same area as do the ramifications of the cell dendrites. It is worth noting that all the terminals of the thick accessory nerve seem to be limited to this area only as no branches have been found to the muscle itself; in this respect the innervation of the median MRO would differ from that of the lateral one.

As for the relations of the accessory nerves to the processes of the nerve-cells I can only say that, as in the abdominal segments, the endings of all these elements are entangled in a dense neuropile-like network. Such pictures as those in fig. 4, B, C, in which the branching of the dendrites is fairly well seen, can be obtained from preparations which have not fully taken up the dye; if the latter is the case, the whole area, especially in the median MRO,

appears deep blue and even the thicker branches are hardly distinguishable (fig. 3, B, F).

Median MRO of the 7th segment. Only one kind of fibres, the motor ones, could be stated to take part in the nerve supply of this MRO. These motor branches derive from nerves of the neighbouring muscle and none of them associates with the axon of the cell. Surprisingly, no accessory nerves could be found with this MRO. The axon appears in the majority of preparations as a solitary fibre and only in some instances one or two thin fibres could be noticed associating with it, but they could not be followed up to the cell. It cannot be affirmed that this MRO does not receive any accessory nerves coming by this or some other route, but they could not be identified beyond doubt in the same preparations in which they were distinctly stained in the other MROs.

Receptor element of the accessory muscle

A nerve-cell is situated between the accessory muscles which sends its processes into one of the two muscles, viz. that which lies nearer to the second head of the lateral thoracico-abdominal muscle (fig. 2, C, MRO VII lat.). It stains readily with methylene blue and after removal of the overlying connective tissue is easily found in the position shown in fig. 7 unless it is lying, as sometimes happens, between the muscle and the epimeral plate. If this is so, the muscle must be pulled aside and fixed in that position. The nerve-cell is smaller than those of the other MRO but has comparatively much longer dendritic processes; after reaching the muscle these break up into numerous filaments ending in oblong areas, some of which fuse into a seemingly continuous network while others, being more distant, appear isolated (fig. 7). Although at first sight they seem to enter into intimate relations with the muscle, they prove to end on the connective tissue fibres which run along the whole length of the muscle in such numbers that in some places it appears completely covered by them; some penetrate even a little deeper, running between the superficial muscle fibres. On separating this connective tissue from the muscles it can be shown that all the ramifications given off by the nerve-cell processes end on this tissue and none on the muscle fibres. I thought at first that fine muscle elements might be mixed with the fibrous tissue, but this assumption had to be discarded after a fruitless search.

It should be remarked that the second accessory muscle does not possess a similarly strong connective tissue sheath.

The axon joins that nerve, running close to the nerve-cell, which belongs to the 7th segment and into which passes the axon of the median MRO of the same segment after its long course across the dorsal muscles.

It is a plausible suggestion that the nerve-cell of the accessory muscle, despite its different appearance and connexions, belongs to the same system of muscle receptors which in the six abdominal and in the last thoracic segments is represented by two units on each side. As will be shown later, corroborative evidence for this is provided by the behaviour of the same element in

alinurus. Therefore the nerve-cell of the accessory muscle has been classi-ed as the lateral receptor of the 7th thoracic segment although it does not t well into the category of the muscle receptor organs defined previously as omposed of a nerve-cell and a special muscle unit.

It might be suggested that the accessory muscle itself should be regarded s the muscle component of the lateral MRO of the 7th segment, being pre-umably in a functional relationship with it. Against this view it could be

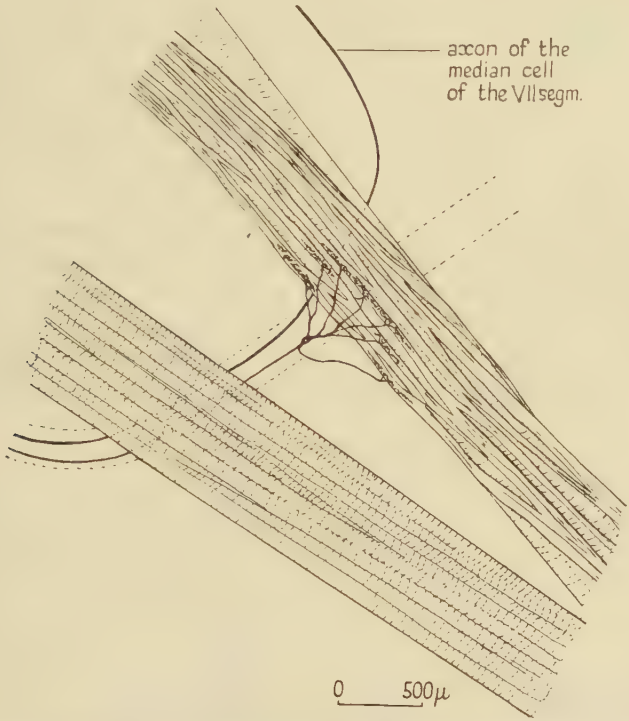

FIG. 7. *Homarus vulgaris*. Semi-diagrammatic view of the receptor cell of the accessory muscle in the position corresponding with fig. 2. The dotted lines indicate the outlines of the nerve of the 7th segment.

rgued that all the receptor muscles hitherto observed exhibit certain charac-eristic features which seem to indicate that they are distinct from the ordinary muscles. Conversely, the accessory muscle in question looks just like an ordinary muscle and apart from its strong connective-tissue coating does not differ in structure from the neighbouring second accessory muscle. Besides, it s much stouter than the muscle components of the receptor organs, and especi-lly considering the smaller size of its nerve-cell its dimensions would appear o be quite disproportionate.

Fibres branch from the nerve trunk running close to the cell and approach he cell and its processes. Owing to the position of the cell and the abundance f nerves in this area, observation of these fibres is somewhat difficult, but in

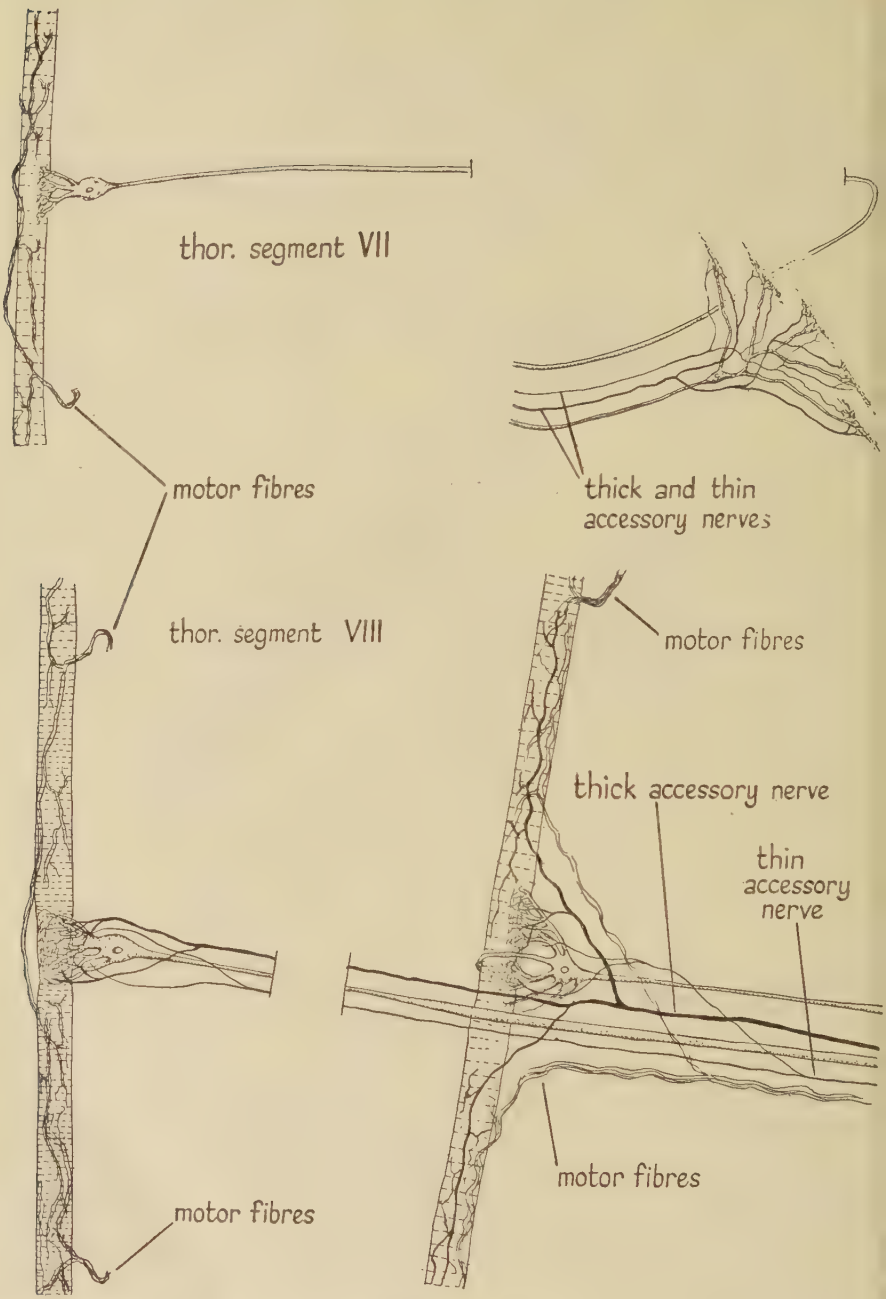

FIG. 8. *Homarus vulgaris*. Diagrams showing the elements of the four muscle receptors o the last two thoracic segments in positions corresponding with those in fig. 2, but at reduced distances.

favourable cases it can be distinctly seen that the cell processes are accompanied by two fibres one of them thicker and the other thinner (fig. 4, D). It must therefore be admitted that both accessory nerves are here represented.

In fig. 8 are given semi-diagrammatic pictures of all the four receptor elements of the two thoracic segments with the nerves described above. Of the latter those only have been included whose individuality and distribution were sufficiently established: any on which there was doubt have been omitted. The main doubts concern two points. First, as regards the two MRO in the 8th segment, more fibres may often be seen taking part in its innervation than are shown in the figure. They run along the axons, associating with accessory nerves, so that each of the latter may be accompanied by one or more fibres. As has been pointed out when dealing with the same problem in the receptor organs of the abdomen, such fibres might be nothing more than the branches of the accessory nerves arising proximally farther away, but one cannot be sure whether this assumption holds good for all similar elements.

The second doubtful point is the absence of the accessory nerves in the median receptor of the 7th segment, which are present in the remaining three. Not having been able to obtain preparations in which their occurrence was unquestionable, I have not shown them in the drawing, although I am not unconscious of the fact that this omission might prove to be a major deficiency in the representation of the receptor elements given above.

Receptor elements in other thoracic muscles (N-cells)

On some muscles inserting in the median surface of the epimeral plate nerve-cells may be seen which presumably have some receptor function, but they differ to such a degree from those previously described that it seems appropriate to place them in a special category. They will be referred to below as N-cells, this conventional term being adopted for reasons that will be explained later.

The N-cells, five in all, have been found at the points indicated by numerals in circles in fig. 2, C. Two of them (Nos. 1 and 2) are situated at the insertion of the second head of the lateral thoracico-abdominal muscle (fig. 10, A). The one lying at the ventral edge of the muscle near to its insertion, as seen on the left side of the figure (No. 1 of fig. 2) often becomes displaced round this edge and then becomes invisible; the other (No. 2) situated at the anterior margin of the muscle may be easily found in almost every preparation. The distance between the two cells may vary and once they were found close to each other.

The cells are comparatively small, not more than half the size of the cells of the MRO, but their dendritic processes, one or two of which may spring from the axon, attain a considerable length (figs. 9, B, 10, B). The dendrites take various courses but all insinuate themselves between the muscle fibres; some may end not so far from the cell (fig. 10, C) but most of them run much farther. Unfortunately, the only processes which stain well are those which run superficially for some distance, e.g. those alongside the attachment of the muscle (fig. 10, A, B); those penetrating deep between the muscle fibres do not

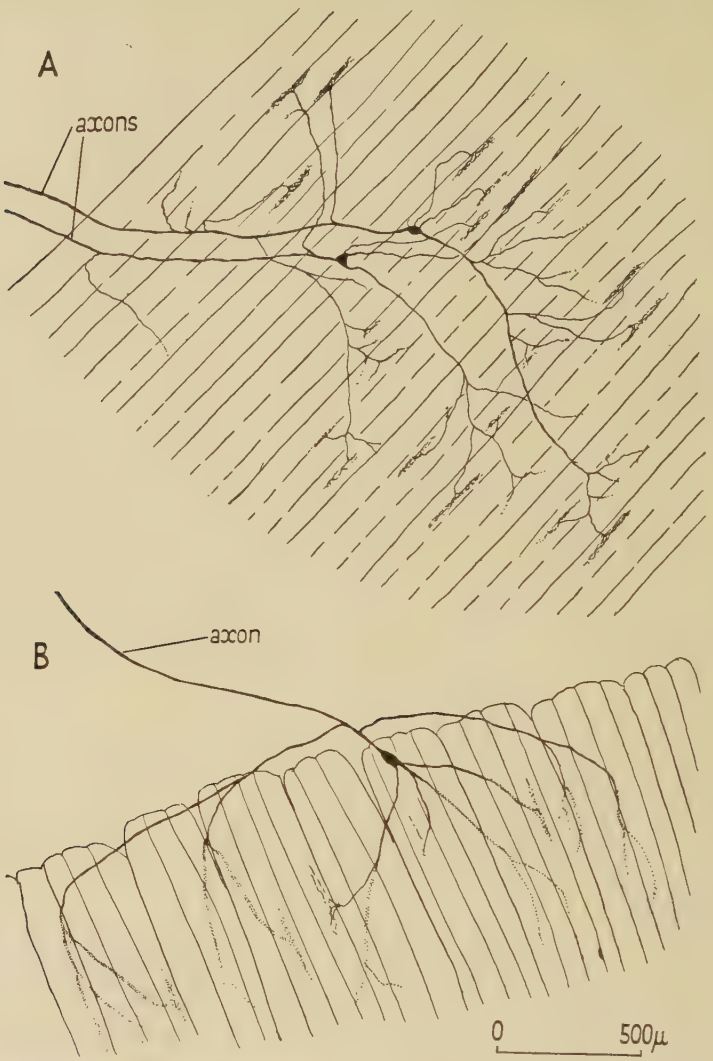

FIG. 9. *Homarus vulgaris.* A. N-cells on the m. attractor epimeralis of the right side (Nos. 4 and 5 of fig. 2); B. N-cell at the insertion of the second head of the lateral thoracico-abdominal muscle of the right side (No. 2 of fig. 2) drawn from several preparations; the dotted lines represent processes travelling deeper within the muscle and which mostly could not be traced up to their terminations.

take up the dye and no evidence could be obtained as to how far they may go. Hence the drawing (fig. 9, B) must be regarded an an illustration merely of what may be seen in preparations and not of the true extent of these elements. From what may be seen of their endings it appears that the fibres break up at a certain point into several branches, spreading their ramifications in the elongated areas between the muscle fibres. Whether they pass on to the muscle tissue, or, as I am rather inclined to believe, are confined to the

interstitial connective tissue, are questions to which no certain answer could be given.

Each axon joins a different nerve trunk; even when the cells were found situated close to one another their axons took different courses, but I was unable to follow them proximally far enough to see whether they belonged to the same segment or, as seems more likely, ran to different ganglia of the nerve cord.

The third N-cell is situated on the ventral edge of the quadrangular m. contractor epimeralis near to its anterior insertion (fig. 2, No. 3). This cell is more difficult to find because of its position, and also because it is often pulled away when the connective tissue covering this muscle, and impeding the staining, is removed. The cell is smaller than the former two but otherwise exhibits similar features (fig. 10, D).

Two other cells lie on the m. attractor epimeralis on that portion of the muscle which inserts near to the dorsal margin of the m. contractor epimeralis (fig. 2, Nos. 4 and 5). It should be noted that this portion is reinforced by an inner layer of muscle fibres taking an oblique course and the nerve-cells are situated on the inner side of these oblique fibres. The cells are small but have exceptionally long processes which may easily be taken for ordinary nerve fibres supplying the muscles (figs. 9, A, 10, E). The sizes of the cells are so out of keeping with the dimensions of their processes that at first I mistook them for accidental enlargements of the motor nerves, and only after I had noticed their repeated occurrence in the same position and examined them more closely could I disclose their true nature. In one preparation three cells were found. I was unable to satisfy myself whether this was an abnormality or whether perhaps it was an element which is normally situated more deeply but happened to lie nearer to the surface and could then be detected.

The processes can be followed farther than those of the cells previously described owing to the thinness of the muscle on which they are lying, but even so many of them cannot be traced to their terminations, and I have reasons to believe that if all branches had been stained the territory they occupy would be much larger than represented in the figure. The same may be said about the areas of expansion of the terminal fibres: because they break up into numerous filaments, and because the picture is also confused by the fibres of motor nerves, the limits of the terminal areas are uncertain and their length is probably greater than is shown in the figure.

Processes of the same appearance as those given off by the cell-bodies arise from their axons also and it is surprising to see them branching even at the points of the axon situated nearly 1 mm. from the cell. Their number is variable, seven being the highest observed, but as some are possibly not stained in methylene-blue preparations they may be in fact even more numerous. They run in various directions, penetrate the muscles, and terminate between the muscle fibres exactly in the same way as do the branches of the cell dendrites. It should be emphasized that nothing could be observed indicating that they might be of a different sort. It would therefore appear that,

considering the N-cells to be sensory elements, the stimuli received by their terminals are conveyed not only to the cell-bodies but to their axons as well.

The axons pass into a nerve running ventrally along the anterior margin of the m. contractor epimeralis.

Judging from the courses taken by the axons of the five N-cells it may be assumed that they enter the first three thoracic ganglia and possibly also the suboesophageal ganglionic mass, but the exact relations could not be satisfactorily demonstrated. Difficulties in observing the nerves on the epimeral plate are increased by the fact that both the pericardium and also some of the so-called heart ligaments have their attachments here, and both have extraordinarily abundant nerve-supplies. They obstruct the view when left *in situ* and therefore have to be removed, but during their removal the cell axons and even the cells themselves may be easily damaged.

Palinurus vulgaris

Topography of the dorsal thoracic muscles

The general arrangement of the thoracico-abdominal muscles as they come to view in the first stage of their dissection is basically the same as in *Homarus* and thus in the drawing representing these muscles in fig. 1 only slight modifications would be needed for *Palinurus*. In further stages of dissection, when the muscles are cut out and the median surface of the epimeral plate is exposed to view, some differences are noticeable (fig. 11). Of those relating to the dorsal thoracico-abdominal muscles, which are of minor importance, the following may be mentioned: the distance between the anterior and posterior insertions of the third dorsal muscle is comparatively shorter in relation to the length of the curving fibres so that on the flattened preparations they are seen to take a semicircular course; the differences in the obliquity of the fibres of the second and third dorsal muscles are less accentuated than in *Homarus*. The same slight difference in colour observed in *Homarus* is noticeable between the first and second muscle on the one hand, and the third on the other, and on histological examination similar features have been observed in their cross-striation.

Fig. 10 (plate). Receptor elements of the second category (N-cells) in *Homarus vulgaris*. The scale below E applies also to B, C, and D. (Photomicrographs made from preparations stained with methylene blue, fixed with ammonium molybdate, and mounted in xylene dammar.)

A. N-cells on the second head of the lateral thoracico-abdominal muscle of the right side (Nos. 1 and 2 of fig. 2). The axon of the cell No. 1 passes into a nerve trunk. In the upper right corner of the figure a part of the m. contractor epimeralis may be seen.

B. N-cell at the insertion of the second head of the lateral thoracico-abdominal muscle (No. 2 of fig. 2) of the left side with a long process springing from the axon.

C. N-cell on the second head of the lateral thoracico-abdominal muscle (No. 1 of fig. 2). Its axon joins the nerve trunk passing near-by.

D. N-cell on the m. contractor epimeralis (No. 3 of fig. 2).

E. N-cell on the m. attractor epimeralis. (Nos. 4 and 5 of fig. 2). Note the processes arising from the axons.

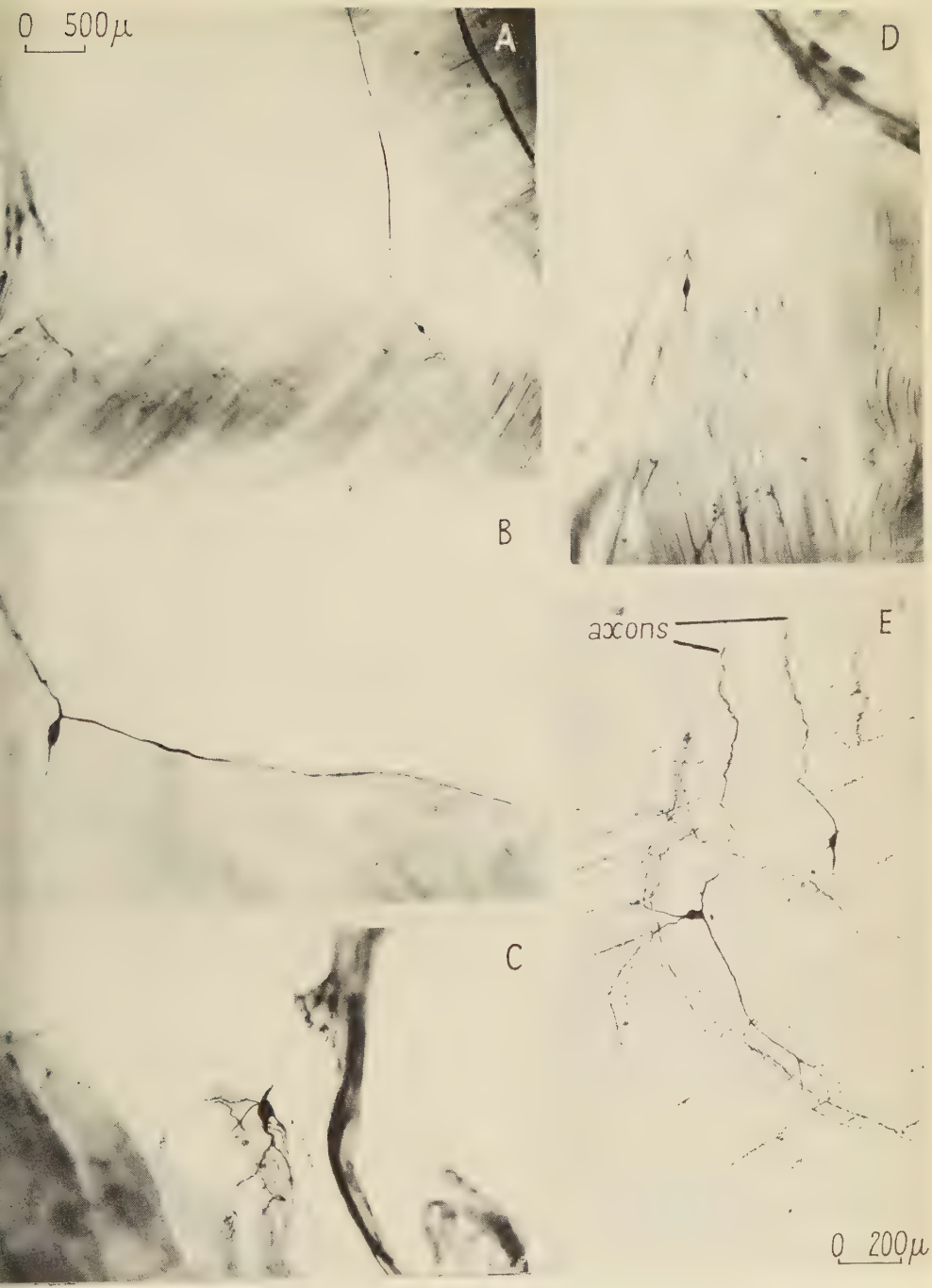

FIG. 10

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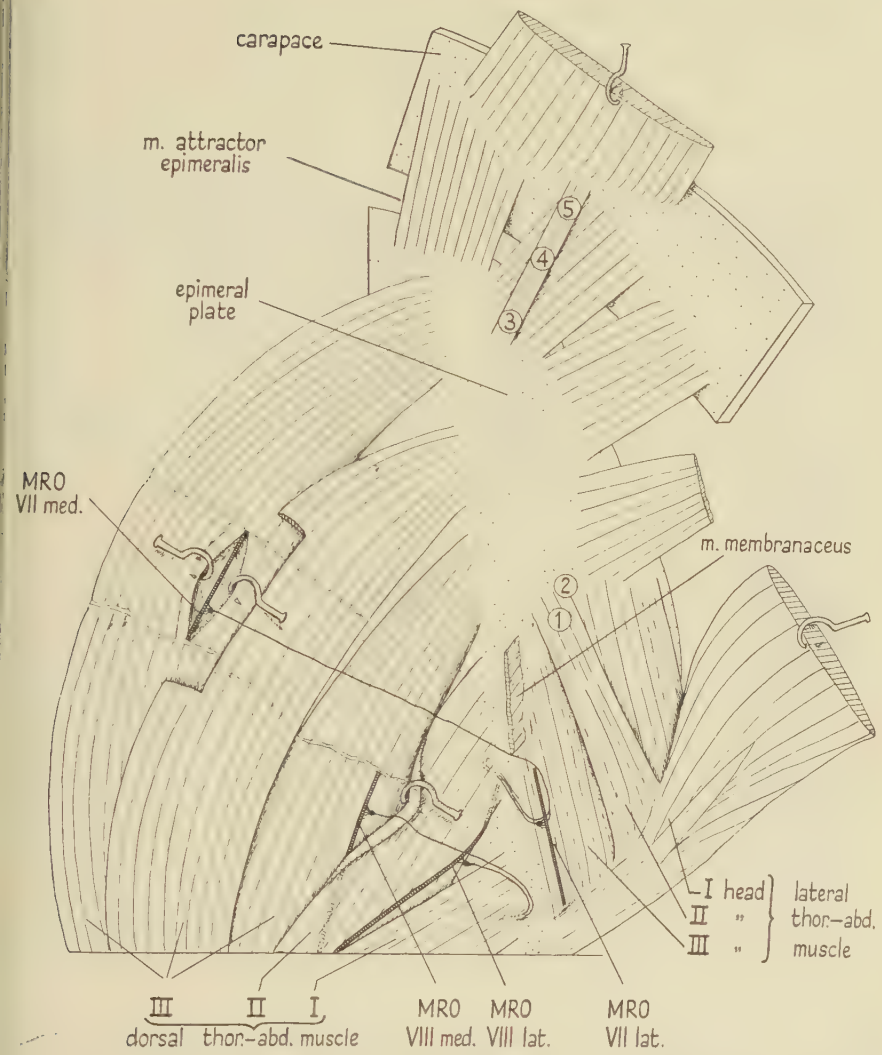


FIG. 11. *Palinurus vulgaris*. Muscles of the dorsal part of the thorax of the right side viewed n a preparation spread in the same way as shown in fig. 2. The curved course of the third lorsal thoracico-abdominal muscle results from the flattening of its fibres which in their ormal situation bend round the convexity of the body. For comparison with *Homarus* fig. 2) the following particulars should be noted: different shape of the portions of the lateral horacico-abdominal muscle inserting into epimeral plate; absence of the m. contractor pimeralis; reduction to a ribbon-like strand of muscle fibres of the portion of m. attractor pimeralis in which the N-cells are situated; differences in the situation of the lateral muscle eceptor organs of the 7th and 8th segments (MRO VII, VIII lat.) and of the N-cells, the latter indicated by the encircled numbers 1–5.

Major differences between the two species consist in the arrangement other muscles inserting in the epimeral plate: m. contractor epimeralis totally absent; instead of fan-like diverging portions of the lateral thoracic abdominal muscles (i.e. the second head and the two accessory muscles) *Homarus*, there is in *Palinurus* a bulky muscle mass occupying the posteri part of the epimeral plate stretching ventrally up to the insertions of the m membranaceus and of the first dorsal thoracico-abdominal muscle. In th muscle mass a superficial portion shows a certain independence in its attach ments and its fibres have a slightly different course; this portion will b referred to as the second head and the rest as the third head of the later thoracico-abdominal muscle.

Muscle receptor organs in the 7th and 8th thoracic segments

As in *Homarus* the two MRO of the 8th segment and the median one the 7th segment are topographically in connexion with the dorsal thoracic abdominal muscles. The lateral MRO of the 7th segment lies at the thir head of the lateral thoracico-abdominal muscle.

Receptor organs in the dorsal thoracico-abdominal muscles

The two MRO in the 8th segment are farther apart from one another tha in *Homarus* (fig. 11). The lateral one is situated on the median edge of th first dorsal muscle and its cell lies near the anterior insertion of its inne fibres. The receptor muscle has its origin close to the attachments of the inne fibres of the first dorsal muscle, but in its forward course it comes to lie ove the second dorsal muscle and inserts among the fibres of the latter into th epimeral plate. The portion of the receptor muscle lying in front of its nerve cell is in reality much longer than it appears to be at first sight, for curvin round the convexity of the second dorsal muscle it can only be seen in i whole length after the preparations are well stretched.

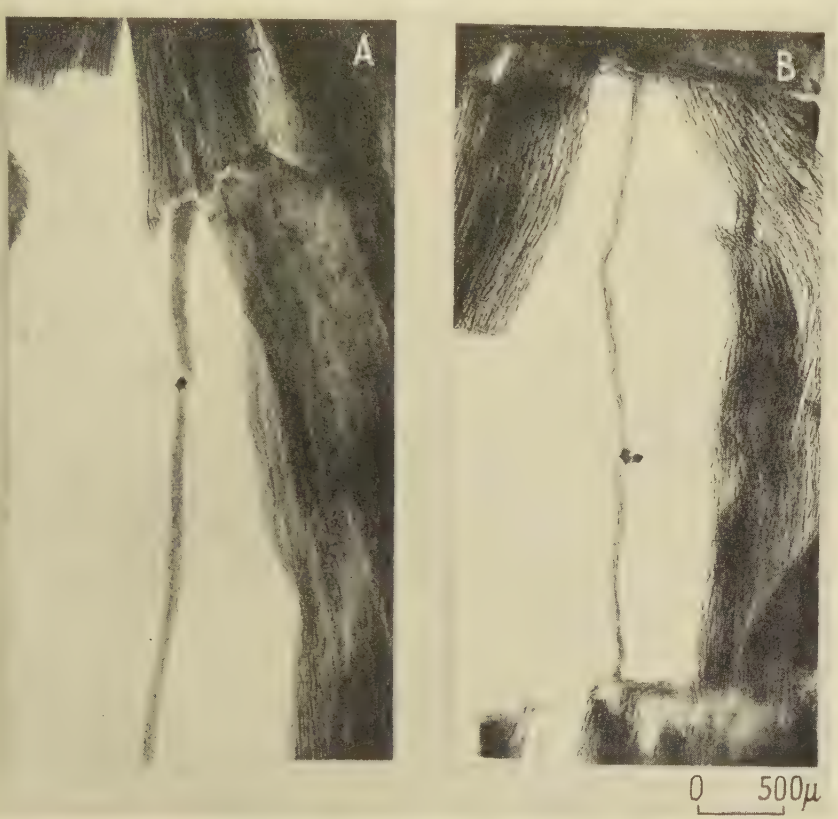
The muscle of the median MRO of the 8th segment has its posteric attachment among those fibres of the third dorsal muscle which are ove lapped by the second dorsal muscle. In its forward course it comes nearer t the surface but it can only occasionally be seen without removing or pushin aside the overlying muscle fibres. Its anterior insertion is not far from th nerve-cell, in the connective tissue intersecting the bundles of the third dors thoracico-abdominal muscle (figs. 11 and 12, A).

The muscle of the median MRO of the 7th segment is situated deep among the bundles of the inner portion of the third dorsal muscle. Both i ends are attached to the fibrous intersections of the muscle fibres (figs. 11 an 12, B). The position of the nerve-cell is variable, but it has always been foun to be nearer the posterior end of the muscle.

Structure of the muscles

Each of the three muscles has its characteristic features. The lateral or of the 8th segment is the longest of all. It carries a large amount of den

onnective tissue which in its posterior half forms a sheath gradually increas-g in thickness and predominating over the muscle tissue. In transverse sec-ons it can be seen that this sheath attains a considerable thickness on both anks of the muscle but is much thinner on its dorsal and ventral side. The oss-striation of the myofibrils is of the coarser type.



IG. 12. *Palinurus vulgaris*. Photomicrographs showing in A, the median receptor organ of ie 8th segment of the left side inserting into a tendinous intersection of the third dorsal ioracico-abdominal muscle; in B, median receptor organ of the 7th segment of the right de with both attachments of its muscle to the tendinous intersections of the third dorsal thoracico-abdominal muscle.

The muscle of the median MRO of the 8th segment is shorter than the ormer. Having only a little surrounding connective tissue, it is nevertheless nicker than the lateral one with its sheath and thus proves to have more of nuscle tissue, the relation between the two being in *Palinurus* the reverse of nat in *Homarus*. Histologically, the median muscle of the 8th segment is naracterized by thin myofibrils with fine cross-striation.

The muscle of the median MRO of the 7th segment is the shortest of the nree and is accompanied only by sparse connective tissue elements. Other-ise in its structure it is more like the lateral muscle of the 8th segment,

having cross-striations of a coarse type. This was unexpected since the media receptor organs in the thorax must be considered as corresponding to thos in the abdomen described under the name of receptor muscles 2 (RM 2), a of which are of the finely striated type.

All three muscles have intercalated connective tissue occupying the whol thickness of the muscles at the areas of the distribution of the cell-dendrites Should myofibrils pass uninterruptedly through this area they will only b a few single elements, and not bundles such as are in *Homarus* in the later MRO of the 8th segment and the median one of the 7th.

Nerve-cells

The nerve-cells of all three MRO are of about the same size as those i the abdominal segments and resemble them in other details such as the shap of the cell-body and the mode of branching of the processes. The difference between the arrangement of the dendrites of the lateral cell of the 8th seg ment on the one hand, and of the two median ones on the other, is not s marked as in *Homarus*. All cells are more spheroidal in shape and more un form in their appearance (fig. 13, A, B). They are enclosed in capsules and th latter are encircled by concentric layers of connective tissue. A network o fine nerve filaments may be observed, although not often, around the media cell of the 8th segment. A similar basketwork is presumably also preser round the median cell of the 7th segment, but it is rarely and indistinctl stained.

The axons of the cells do not show such excessive enlargements of the diameters as in *Homarus*.

Nerves

The arrangement of the nerves supplying the three MRO is on the sam lines as in *Homarus*. A slight difference in the motor innervation of the later

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FIG. 13 (plate). Receptor elements in the thoracic muscles of *Palinurus vulgaris*. All phot micrographs were made at the same magnification from preparations stained with methyle blue, fixed with ammonium molybdate, and mounted in xylene dammar.

A. Lateral muscle receptor organ of the 8th segment of the left side. The branches of t two accessory nerves approach the dendrites of the cell; on the extreme left a branch of t thick accessory nerve runs to the receptor muscle; the bundle of nerve fibres on the extrer right carries the motor nerves and a branch of the thick accessory nerve.

B. Median receptor organ of the 8th segment from the same preparation as the lateral o in the foregoing figure; from the two accessory nerves only the thick one is clearly visibl beneath the receptor muscle runs the motor fibre. Note the constriction of the muscle at t region of the intercalated tendon.

C. Lateral receptor organ of the 7th segment. Note the long and widely expanding pr cesses of the nerve-cell.

D. N-cell on the second head of the lateral thoracico-abdominal muscle (No. 1 of fig. 1 with one process giving off several short branches ending into lamelliform areas.

E. Same cell as in D, from another specimen.

F. N-cell on the m. attractor epimeralis (No. 5 of fig. 11). The long process running upwar is one of the dendrites which could be traced up to the insertion of the muscle fibres; or a short part of the axon is stained.

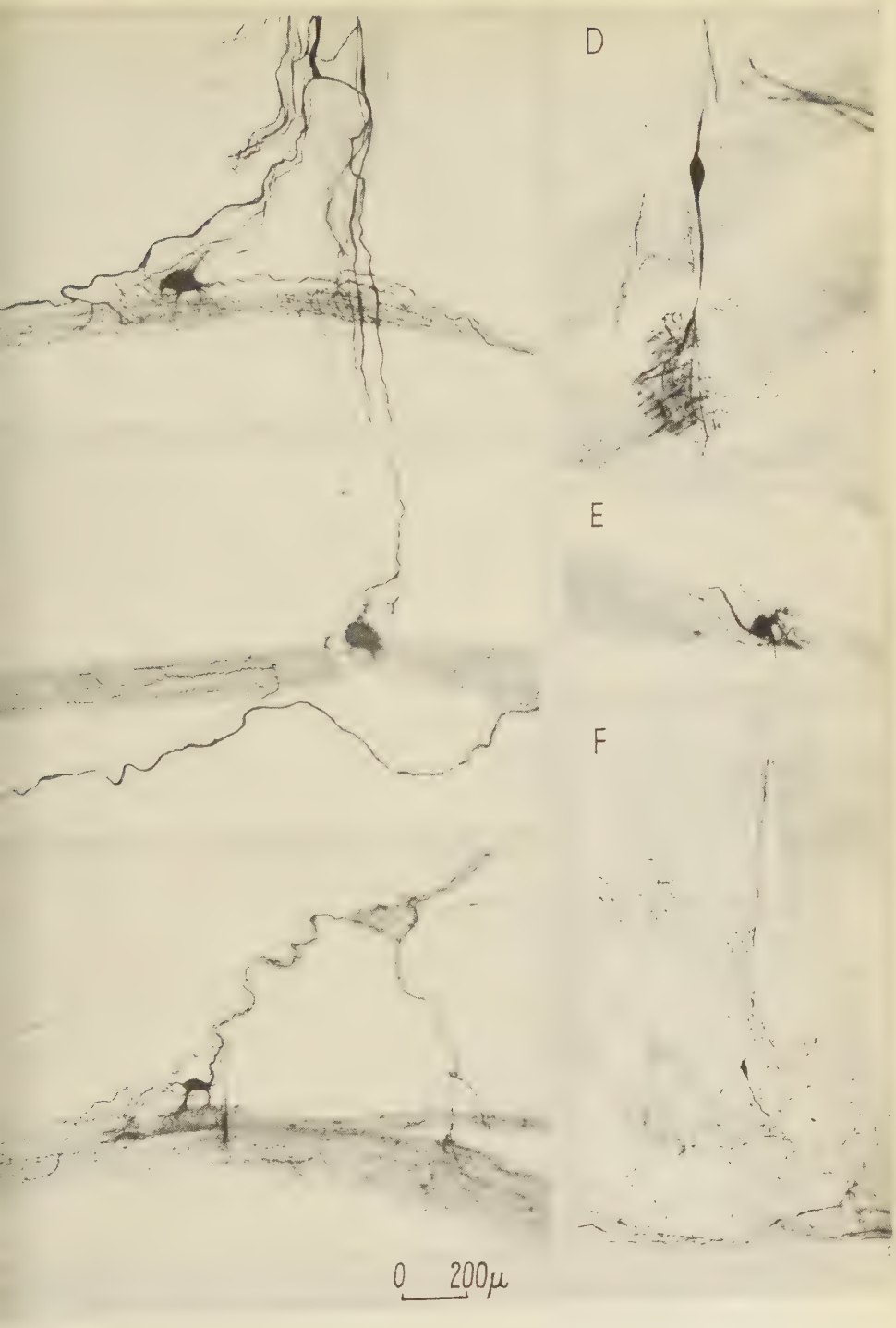


Fig. 13

J. S. ALEXANDROWICZ

MRO of the 8th segment is noticeable in that one stouter fibre runs along the ell axon, justifying the name of the main motor fibre; the other 'additional' notor fibres are also present. The two median MRO receive their motor nnervation exclusively from branches given off by the nerves of the neighbouring muscles. The photomicrograph (fig. 13, B) shows a motor nerve running alongside the muscle of the median MRO of the 8th segment and sending ranches to this muscle.

The two accessory nerves differ in their calibre even more than in *Homarus*. articularly remarkable is the thickness of the branch of the accessory nerve unning to the lateral receptor muscle of the 8th segment (fig. 13, A, fibre on he left side of the cell). In the median MRO of the same segment the branching of the accessory nerves has been observed only in the area of the terminations of the cell dendrites.

An accessory innervation of the median MRO of the 7th segment could ot be seen. Some doubts on this point arose from the nerve fibres accompanying the cell axon but in so far as they could be traced they proved to be estined for ordinary muscles.

ateral muscle receptor organ of the 7th thoracic segment

The lateral MRO of the 7th segment is situated on the ventral edge of the hird head of the lateral thoracico-abdominal muscle. Its muscle component uns in the same direction as the fibres over which it is lying, but shows its ndividuality in its thinner and more finely cross-striated myofibril bundles; t sometimes also exhibits different staining properties, taking a deep blue olour when other muscles are only faintly stained. There are a good many connective tissue fibres running longitudinally with this muscle and in transerse sections it can be seen that they run not only on the periphery but lso between the bundles of myofibrils.

The nerve-cell is smaller than those in the three other MRO and more rregular in its shape. In some preparations it may look quite like one of the ther MROs but more often has its individual and variable features (fig. 3, C). Its processes are as a rule longer and often arise from the axon too. They approach the muscle at points more widely spread and apparently end n the connective tissue fibres running amongst the myofibril bundles.

The axon passes into the nerve which carries the axon of the median cell f the same segment.

Several nerve fibres reach this MRO ramifying in the muscle and around he cell dendrites. Owing to technical difficulties the preparations were less atisfactory for determining the distribution of these elements to the same legree of exactness as in other MRO. The best evidence indicates that all hree kinds of nerves, i.e. the motor and both accessory nerves, are present.

Hints on displaying the receptor organs

In order to get all the MRO stained the preparations must be spread as hown in fig. 11 and one has at first to wait until the lateral MRO of the

8th segment, which is superficially situated, becomes stained just enough t be visible. The nerve trunk which crosses this MRO in front or above it nerve-cell carries the axon of the median cell of the 8th segment and therefor if one follows this axon one may arrive at the area where its cell is situated The median MRO of the 7th segment may be found in the same way, i.e by tracing its axon which must be looked for in a strand of tissue passin near the insertion of the inner fibres of the second dorsal muscle to th epimeral plate. In both cases only the approximate position of the cells ma be guessed before they take up the dye.

In *Palinurus* the search for the median MRO of the 7th segment is mor difficult than in *Homarus* since its axon is accompanied by ordinary nerve and some experience is needed until one learns to spot the axon and follo it to the right place, and even then occasional mishaps are likely to occu Once, however, the nerve-cell becomes noticeable the tracing of the recepto muscle is much easier; preparations such as those represented in fig. 12, B i which the whole muscle up to its attachment has been exposed to view, could obtain only from *Palinurus*.

The lateral MRO of the 7th segment is not easy to find. It lies at the ver edge of the muscle and this is situated more deeply than one is likely t assume after having stretched the preparation as in fig. 11. The groove be tween the first dorsal thoracico-abdominal muscle and the third head of th lateral thoracico-abdominal muscle is covered by connective tissue and thi has to be removed, but in doing so one can easily tear away the nerve-cell It is therefore advisable to proceed by steps, viz. to remove a layer of th connective tissue, leave the preparation in the staining solution, and examin after some time. This can be repeated till the nerve-cell is spotted. Even afte some experience one cannot always be sure of success.

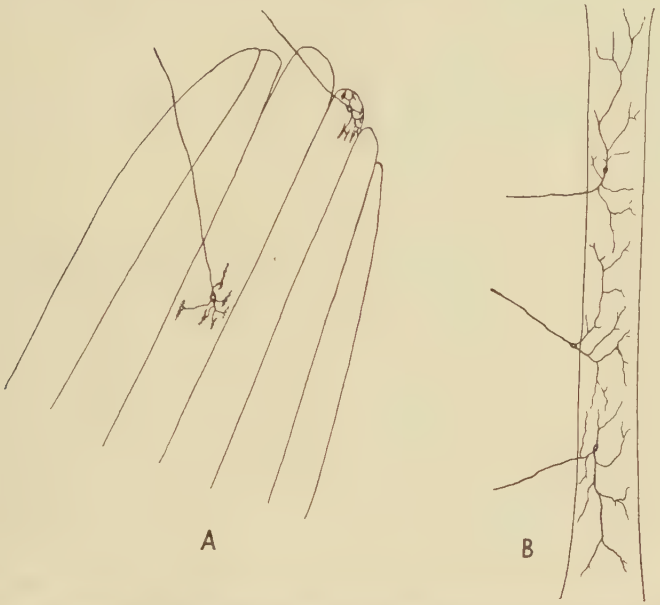
Receptor elements in other thoracic muscles (N-cells)

Receptor elements of the second category (N-cells) have been observed i *Palinurus* on the second head of the lateral thoracico-abdominal muscle an on the m. attractor epimeralis (fig. 11, Nos. 1–5).

The cells on the second head of the lateral thoracico-abdominal muscle ar two in number and are situated one on its median surface not far from th insertion of this muscle but always at a certain distance from it, the second a the insertion of the dorsal fibres in the epimeral plate (fig. 14, A). They ar evidently the same elements which have been found in *Homarus* at about th same place and this has provided an argument for seeing in the muscle bundl with which they are connected a homologue of the second head of the latera thoracico-abdominal muscle in *Homarus*, although these portions of the muscl differ markedly in size in the two species.

The nerve-cells show great variety in their appearance from different speci mens, so that if they were not observed at the same spot one might think tha they are not of the same kind (fig. 13, D, E). Their dendrites spring from variou points and vary in number; in some cases only one stout distal process ma

e present. They are of variable length: some end in the vicinity of the cell d then their terminations can be better seen (fig. 13, D); this photomicro-aph shows the abundance of the terminal branches, but to get a true idea their extent one has to remember that the dark parallel lines seen in the gure are the edges of the small lamelliform terminal areas extending be-veen the muscle fibres. Although such endings are rarely seen it seems



IG. 14. *Palinurus vulgaris*. Diagrams showing the position of the N-cells on the second head the lateral thoracico-abdominal muscle of the right side (A), and on the strand of fibres of m. attractor epimeralis of the right side (B). Cf. fig. 11, Nos. 1–5.

robable that all the processes end in a similar way (perhaps not all hav-g so many terminal areas at one place).

The axons of the cells associate with the nerves running near-by.

The cells of the m. attractor epimeralis lie on that portion of this muscle hich is near to the anterior attachment of the first head of the lateral oracico-abdominal muscle and which has on its median side an additional in bundle of muscle fibres. The latter has a slightly oblique direction and serts with both its ends a little beyond the line of attachment of other tractor fibres (fig. 11). On this ribbon-shaped strand of muscle fibres three erve-cells are situated, one in the middle and the two others nearer its ends g. 14, B). These are small elements of variable shape with very long processes hich, as in *Homarus*, may arise from the axon. They expand so far from the lls that the areas of their distribution practically cover the whole muscle, some places even overlapping each other. As seen in the photomicrograph g. 13, F) the cell processes might easily be mistaken for ordinary motor

nerves, especially since they associate with the branches of these nerves run ning to the muscle. In favourable cases only it may be noticed that there is certain difference in the arrangement of their filaments, but as a rule th discrimination is so uncertain that unless a fibre can be traced up to the ce it is impossible to recognize it as the branch of the cell process.

The cells themselves stain usually uniformly dark blue, but when som happen to take a paler hue each may be seen to have a nucleus, and there nothing in their appearance which could support any doubt about their bein true nerve-cells.

The axons enter the nerve branches passing in the vicinity. As to their fina destination, as well as of those of the two other N-cells, the same reservatio must be held as before for the similar elements in *Homarus*.

Discussion

In an attempt to classify all the muscle receptors of crustaceans so fa investigated into well-defined groups one meets with difficulties because th available evidence is based only on the morphology of these organs, leavin many problems to be answered. At any rate it seems justifiable to assume tha there are two main categories: one including the MRO of the abdomen an the two posterior thoracic segments, the other the elements designated a N-cells. As regards the receptors of the first category they have been defined in the description of the MRO in the abdomen, as consisting of a nerve-cel connected with a special muscle unit. However, this definition, which in simple way could establish the distinction between receptor organs having muscle of their own and receptor cells ending in ordinary muscles, woul exclude the lateral cell of the 7th segment in *Homarus* from among the ele ments of the first category with which, in all probability, it forms one an the same system. Therefore, the definition given above requires an addendu to the effect that in some cases a receptor element presumably belonging t the same functional system may not have a muscle of its own. As yet onl one such exception has been recorded, but it is conceivable that in some othe species at the same or some other place the muscle component might b missing.

An interesting feature of the MRO of this category is that they are of tw different types in each segment. It may be recalled that in the abdomen o *Homarus* such differences have been observed and in describing them it ha been pointed out that the dendrites of one cell, termed cell 1, occupy a large area with less regular outlines whereas the area of terminations of the secon cell (cell 2) is smaller and more sharply delimited. These differences may no be great in *Homarus* and may be even less conspicuous in *Palinurus*, but in th Paguridae (*Pagurus striatus*, *P. calidus*) they are so obvious that the existenc of the two types of elements in these animals is beyond doubt. Hence it ma be inferred that the same is the case in the other species though the differenc in the external appearance of the nerve-cells may be less accentuated in on species than in the other and also in different segments of the same specie

In the thoracic segments of *Homarus* each of the receptor cells shows eculiarities of the same kind as in the abdomen, and the differences between he two elements of the same segment are even more pronounced than beween those in the abdomen. It may therefore be concluded that the two lateral IROs in the thorax belong to the same sort as the cells 1 of the MROs in the bdomen—they all may be called cells of the first type; and accordingly the nedian cells of the thorax and the cells 2 of the abdomen may be distinguished s cells of the second type.

Comparison of these cells in the various segments and in different species hows that the cells of the first type exhibit much greater diversity of form. Thus in *Palinurus* their dendrites are not much longer than in the cells of he second type and only the lateral cell of the 7th thoracic segment has onger and more widely spread processes; in *Homarus*, as has been pointed ut, the lateral cells have a characteristic and variable appearance and that n the 7th thoracic segment is quite unlike the others; in *Pagurus* the cells of his type develop strikingly long processes extending along the receptor nuscle. In all these instances the cells of the second type, though far from eing uniform, deviate much less in their shape from the common type haracterized by short processes with branches forming a dense tuft ending n a comparatively small area of the receptor muscle.

In the description of the abdominal MRO it has been stated that the nuscles of the two receptors in each segment have a different structure, that elonging to the cell 1, i.e. of the first type, having thicker myofibrils with coarser cross-striation than the other. The same differences have been found n the 8th thoracic segment. But this was not so in the 7th segment of *Palinurus*, s the muscle of the median MRO has coarser cross-striation than that of the ateral one. Consequently, the type of cross-striation cannot be regarded as eing linked in all instances with the nerve-cells of one and the same type. At this point it may be remarked that the ordinary muscles of these crustaeans also show differences in their cross-striation.

The nerve supply of both median MRO in the thorax has particular eatures worthy of consideration. The difference in the courses of the motor ibres does not raise any problem: it merely shows that the receptor cell and he motor nerves, during their development, come into relation with the eceptor muscle by independent paths. Of more importance is the behaviour f the thick accessory nerve. In the 8th thoracic segment it gives off branches o the cell dendrites and to the muscle of the lateral MRO, but in the median MRO it is restricted to the cell dendrites only. This would mean that, if this erve carries, as one may assume, either excitatory or depressing impulses, n one case these impulses would be transmitted simultaneously to the nervecell and to the whole muscle (presumably to its nerves), in the other case to he cell only. In the representation of the connexions of this nerve in the bdominal segments it has been assumed that it supplies both MRO in an qual way, i.e. the muscle of the second MRO as well. To explain this discrepancy, if one is not inclined to admit such a difference in the nerve supply

of the receptor organs in the various segments, it may be conjectured that the 8th thoracic segment the accessory fibres to the muscle of the media receptor take a different route from those to the cell, viz. run with the moto fibres, but it is also possible that the interpretation in my previous paper w an erroneous one resulting from confusion of the nerve fibres in the close associated muscles. If this be so, the pattern of distribution of the thick accesso nerve, such as has been observed in the 8th thoracic segment, should perha hold for the abdominal segments too.

As to the absence of the accessory nerves in the median MRO of the 7t segment, various suppositions can be made: they may possibly run with th cell axon but remain unstained, or else they may go by the same route as th motor nerves, or, finally, they may be in fact lacking. Direct observatio seems to support the latter supposition, but, as was said before, the evidenc is not convincing.

A question arises whether the receptor elements of this category are con fined to the abdomen and the posterior part of the thorax, or whether the are also present more anteriorly. I am inclined to favour the former view, bu caution is advisable in making this statement since it is found that these organ may be completely surrounded by the ordinary muscle fibres, as, for example the median MRO of the 7th segment. The latter was discovered when, seein a long solitary nerve fibre running across the muscles and not giving o branches to them, I suspected that it might be of some special character; bu if this cell-axon had run under the muscles together with the motor fibres the chances of finding this MRO would have been much less.

I tried to obtain some information as to possible occurrence of more MR in the thorax from preparations of the embryonic lobster. In these it wa possible to follow the axons of the abdominal receptor cells into the ganglion cord where they form a special tract running through all the abdominal an thoracic segments (Alexandrowicz, 1951). Observation of the MRO in th thorax is difficult, since other elements staining with methylene blue mak the picture confused; but it can be seen that the tract of MRO is joined in th last two thoracic segments by fibres similar to those in the abdomen and b no such fibres more anteriorly. This provides corroborative evidence for th assumption that the muscle receptor organs of this category number onl four on each side of the thorax.

The N-cells are diverse in their appearance and any name referring to thei shape and also to their situation such as small cells, or cells with long den drites or cells situated at the insertion of the muscles, would be unsuitable To understand why they are there the following questions should be answered (1) Are similar elements more widely distributed, or are they confined to th thoracic muscles? (2) Are they of one or different sorts? (3) What may thei function be? To none of these questions can a satisfactory answer as yet b given. The occurrence of the nerve-cells in other muscles has so far not bee proved. Small swellings can be seen on fibres innervating various muscles c crustaceans which I have suspected to be nerve-cells, but this could not b

ablished with sufficient certainty. The fact that such cells have not been orded, although the innervation of the muscles of crustaceans has been eatedly examined, does not strengthen the supposition that they are every- ere present. On the other hand, it must be recalled that in some other thropoda nerve-cells ending in muscles have been noticed (Hilton, 1924; gosina, 1928) and that their detection depends entirely on their chance posure to the stain at that part of the muscle which is being examined. wever, the fortuitous coincidence which brought to my notice their exis- ce on the epimeral plate might not have occurred elsewhere.

Whether or no all the elements described as N-cells are of the same kind doubtful. Those situated on the second head of the lateral thoracico- dominal muscle certainly look different from those on the m. attractor meralis, but the same elements show such a multiformity in the same species d may be so unlike in the two species investigated that no conclusion can drawn from their appearance alone.

When comparing receptor elements of the two categories, i.e. the MRO of e extensor muscles with the N-cells, one cannot be unaware that, although e differences between them are so pronounced that the establishment of the o categories seems to be fully justified, there are in each of them elements hibiting features which may be regarded as transitory. Thus, on the one nd, the lateral cell of the 7th segment in *Homarus* has not its own muscle; d, on the other, the three cells of the m. attractor epimeralis in *Palinurus* e connected with a particular muscle strand which, though not showing the aracteristic features of the receptor muscle, is separated from the other uscles and is pervaded throughout by the terminations of receptor cells.

All the described forms of the muscle receptors in crustaceans may be garded as representing different stages of evolution of these elements: the ost primitive are obviously the single nerve-cells ending in the ordinary uscles, the latter not showing any particular changes. In the more advanced ge there is a concentration of receptor cells on a comparatively feeble uscle. The two phases of this process are well illustrated by the behaviour that portion of the attractor bundles which carries the N-cells and which, ger in *Homarus*, becomes greatly reduced in *Palinurus*. The highest form the muscle receptors may be seen in MRO of the extensor muscles consist- g of a nerve-cell, a special muscle unit with intercalated tendon, and a mplicated nerve supply. As we have seen, among the receptors presumably longing to the latter category, some may be found in which a special muscle it is lacking, as in the lateral MRO of the 8th thoracic segment in *Homarus*. ne can fit this fact into the picture by assuming that the same muscle which one species functions as an ordinary muscle (accessory muscle in *Homarus*) comes in the other, as in *Palinurus*, reduced in size and serves merely as a uscle component of the receptor organ.

The problem of the functions of all these kinds of receptor elements awaits ution. I have suggested that those in the abdominal segments might enter o action during the flipping of the abdomen in the escape reaction of the

animal. As the thoracico-abdominal muscles take part in the movements the abdomen, the presence of similar muscle receptors in the last two thora segments does not conflict with this idea, but as yet it rests on a pur speculative basis and the fact that the receptors of this category are of t sorts in each segment adds further complications to the problem.

Hypotheses regarding the function of the receptor elements of the seco category (N-cells) will depend on whether these prove to have a more gene distribution or to be confined to the thoracic muscles. If the latter, th presence on the muscles inserting in the epimeral plate might perhaps sugg some relation with the mechanism of the action of the pericardium, whi is also attached to this plate. However, until further investigations succe in affording some indication, all such considerations would stand on a ve insecure foundation.

I wish to record my gratitude to Mr. F. S. Russell, F.R.S., for his ki help in preparing the manuscript.

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A Comparison of some Aspects of the Retinae of the Manx Shearwater, Fulmar Petrel, and House Sparrow

By JAMES D. LOCKIE

Summary

Most birds are either diurnal or nocturnal. The Manx shearwater is one of the very f that regularly perform such normal activities as feeding, hunting, and flying by r, and other activities, such as homing to the nest, by night. The retinal structure the Manx shearwater is compared with that of a diurnal passerine (house sparrow) l a diurnal sea-bird (fulmar petrel), in order to determine what features are common sea-birds and what, if any, may be specific adaptations to vision over a wide range of ht intensity. Both sea-birds have a linear area centralis but lack a fovea. The rods the dark-adapted fulmar and shearwater show great difference of shape from those the light-adapted. The shearwater has more rods at the area centralis, a larger rod-raboloid in the dark adapted retina, and a greater ratio of total number of visual cells ganglion nuclei than has the fulmar.

Introduction

THIS is a comparison of the retinal structure of the Manx shearwater (*P. puffinus* (Brünn)), the fulmar petrel (*Fulmarus glacialis* (L.)), and the use sparrow (*Passer domesticus* (L.)).

This problem suggested itself while I was studying the breeding habits of e Manx shearwater on the island of Eigg, Inner Hebrides, during 1949. ockley's observations on Skokholm were confirmed in that, by day, shear-ters were active off-shore, but only at night did they approach or leave the sting area. Nocturnal activity at the site of the colony in the form of flights out the cliff face was greatest when an overcast sky, mist, or rain reduced ht intensity to a minimum (Lockley, 1942). Activity over such a range of ht intensity is uncommon in birds, and nothing is known of the visual echanism by which it is achieved.

No description of the Manx shearwater retina appears to exist, although ood (1917) shows the fundus of a related species, *P. gravis* O'Reilly, as seen rough the ophthalmoscope. In the initial approach to this problem the earwater retina is compared with that of a diurnal sea-bird, on one hand, d a diurnal passerine, on the other, in order to determine what features are mmon to sea-birds and what features, if any, may be specific adaptations to sion over a wide range of light intensity.

Material and Method

The shearwaters, all at least one-year-old birds, were obtained on the island Eigg in July 1949. The fulmars were taken on Unst, Shetland, in July 1950, d the house sparrows at Edinburgh in February 1951.

uarterly Journal of Microscopical Science, Vol. 93, part 3, pp. 347-56, Sept. 1952.]

A single fulmar and house sparrow were shot by day and were therefo light-adapted. A single shearwater, collected from a nesting burrow by d was light-adapted in daylight for 30 minutes before killing.

One fulmar and one sparrow were collected alive and dark-adapted for o hour in a light-tight box. One dark-adapted shearwater was collected duri the night at the nesting colony. This bird was immediately killed and the ey fixed; the operation was carried out by the indirect light of a small torch.

The retinae were fixed in the following way. The birds that were not sh were first killed with chloroform. A slit was cut round the edge of the corn and Bouin's fluid pipetted into the eye without delay. The complete bulb was then excised and placed, whole, in the fixative. Small samples of t retinae were cut with a sharp scalpel: both retina and choroid were read detached from the sclera in one piece. These selected portions of the retin were wax-embedded, both paraffin and ester wax giving good results. Se tions were cut at 10 μ. Heidenhain's iron haematoxylin was used to sta nuclei, and eosin was used as a counter-stain. Thus both light-adapted a dark-adapted eyes received identical treatment from the moment of fixatio

Retinal pigment was bleached by Mayer's method as given by Gatenby a Painter (1937). All pigment in a 10 μ section was bleached in approximate one hour, with little observable distortion.

Definitions of Some Terms used

Fovea. The actual pit or depression in the area centralis (Walls, 1942).

Area centralis. A retinal area within which the retina is so constructed as afford a marked increase in resolving power (Walls, 1942). The ar centralis is visible macroscopically as a pale region owing to the thicke ing of the nuclear layers.

General fundus. The region between the area centralis and the periphery. Th region occupies most of the posterior segment of the eye.

Periphery. The extreme edge of the retina near the ora serrata.

Internal convergence of visual cells. Walls (1942) uses this purely anatomic term to describe the convergence of the nervous connexions of ma visual cells on one or few ganglion cells. Polyak (1941) and others ha shown that whereas only one or few cones connect with one ganglion ce many rods do so.

Cone. A visual cell possessing an oil droplet situated on the distal side of t ellipsoid.

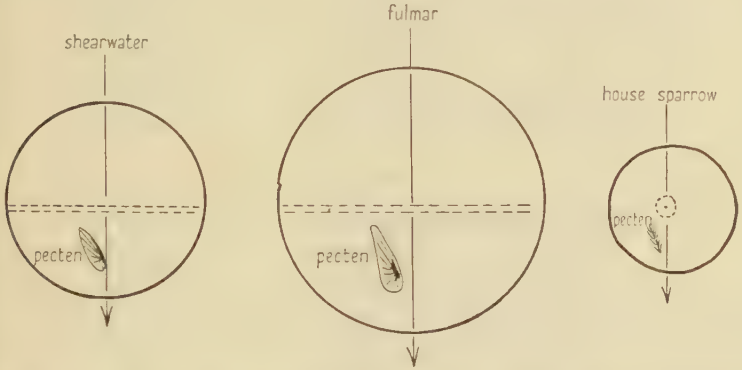
Rod. A visual cell possessing no oil droplet, and under certain known cond tions having a distinct paraboloid, situated on the proximal side of t ellipsoid.

Regions of the Retina

All three species possess a well-defined area centralis, which in both se birds is ribbon-like and below the horizontal axis of the eye, but which in t sparrow is circular and above the axis. All three species have a pure co

ion, but in both fulmar and shearwater this is restricted to a narrow strip, 300 μ, along the centre of the area centralis. In the sparrow this region is ular and much more extensive (fig. 1).

The sparrow has, at the centre of the area centralis, a deep convexiclivate ea with a considerable thinning of the inner nuclear and ganglion layer at point. Neither fulmar nor shearwater possesses a fovea. In the fulmar re occurs a slight thinning of the ganglion layer at the centre of the area tralis (fig. 2).



. 1. Plan view of the retina; the broken line indicates the area centralis; the arrow shows the line along which samples were taken for sectioning.

Anatomy of the Visual Cells

nes

Selected cone cells of the general fundus for the three species are shown in . 3 and 4. They are all similar in form and are typical of the avian cone in sessing a nucleus, myoid, ellipsoid, oil droplet, and slim outer segment. e sparrow cones are in general smaller than those of the sea-birds, par- ularly at the fovea where the outer segments of the cones are rarely pre- ved in sections. The slimness of the cones at the fovea of the sparrow is to correlated with the high concentration of visual cells in this region com- ed with the fulmar and shearwater. In all three retinae the cells tend to ome shorter and plumper towards the periphery. This is typical of all nae (Walls, 1942). Figs. 3 and 4 show a difference in length between dark- pted and light-adapted shearwater and fulmar cones. Some movement of es may take place, but this was not seen to occur in any regular way in the nae examined.

Where cones and rods occur together, the cone nuclei tend to lie nearer the ernal limiting membrane (figs. 3 and 4). However, at the pure cone region ll three retinae the portion of the cone inside the external limiting mem- ne tends to be long and filamentous. This is particularly the case in the rrow fovea where cone nuclei lie some distance from the external limiting mbrane (fig. 2).

Rods

Unlike the cones, the rods of each species differ in several respects a under conditions of light and darkness respectively, assume different shap within a single species.

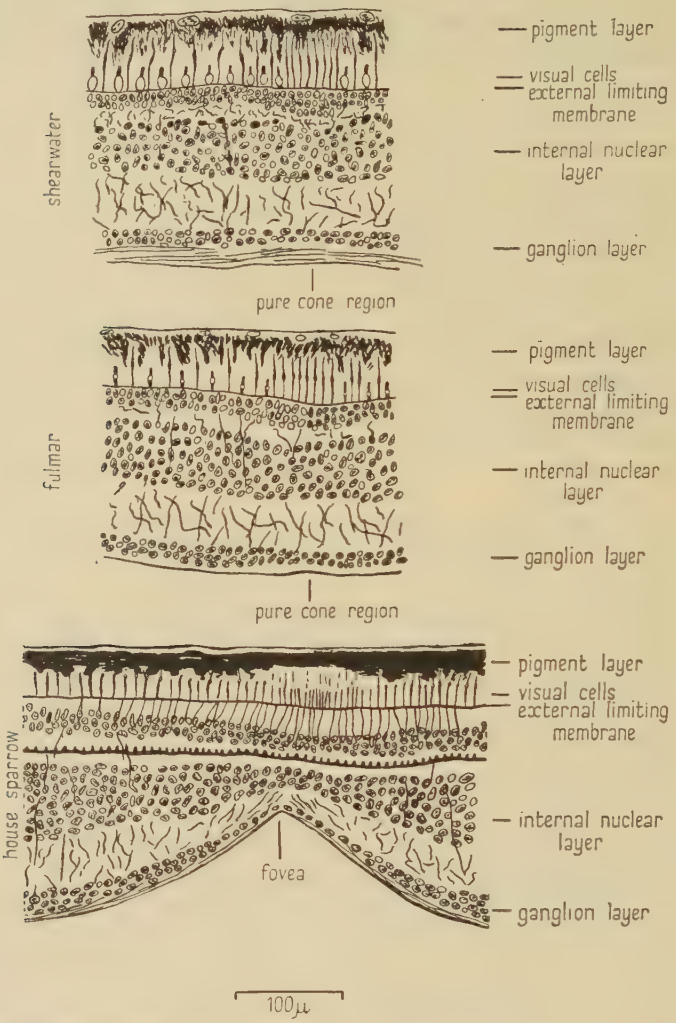
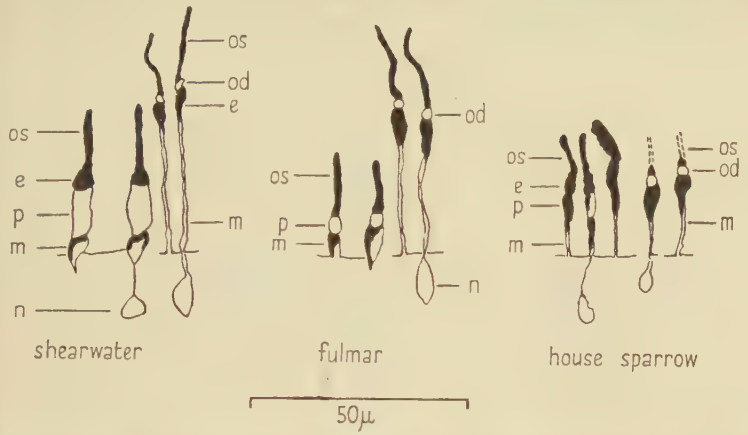


Fig. 2. Sections through the centre of the area centralis in the direction indicated in Fig.

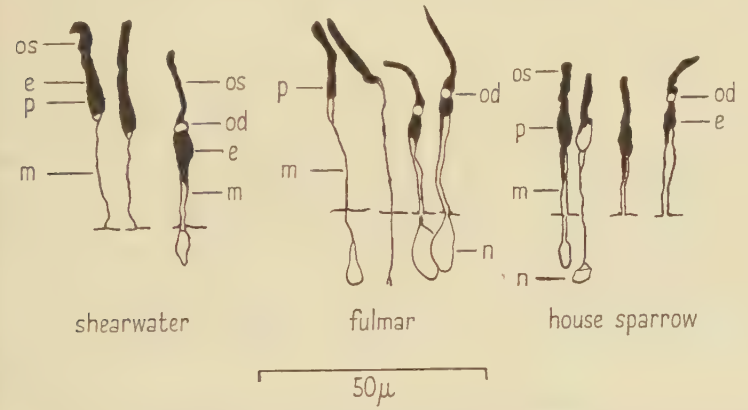
In the rods of dark-adapted shearwaters the paraboloid rests almost on th external limiting membrane and the myoid is thick. The paraboloid is larg and does not stain with either a nuclear or a cytoplasmic stain (fig. 3). Th fulmar rod occupies a similar position to that of the shearwater, relative to th external limiting membrane, but the paraboloid is very much smaller; it als does not stain (fig. 3). Both the shearwater and fulmar paraboloids increase

towards the periphery, but the difference between shearwater and fulmar ains roughly constant.

n contrast, the sparrow appears to possess several types of rods. Those at



. 3. Selected visual cells from the general fundus of dark-adapted retinae. *m* = myoid, = paraboloid, *o.d.* = oil droplet, *e* = ellipsoid, *o.s.* = outer segment, *n* = nuclei. The cells on the left in the case of the shearwater and fulmar, and the three on the left in the case of the house sparrow, are *rods*; the rest are *cones*. Camera lucida drawings.



. 4. Selected visual cells from the general fundus of light-adapted retinae. Lettering ig. 3. The remarks about *rods* and *cones* in the legend to fig. 3 are also applicable here. Camera lucida drawings.

e periphery are the more typical. The paraboloid is usually present and may may not stain deeply (fig. 3).

Whereas rods are easily identified in the dark-adapted retina, their apparent ovement into the pigment layer in the light-adapted retina makes it neces- y to bleach the epithelial pigment in order to make the cells visible.

In light-adapted retinae the rods of both shearwater and fulmar change eir form greatly. The myoid becomes filamentous, the paraboloid is greatly

reduced in size, and the outer segment is carried deep into the pigment lay past the cone oil droplet (fig. 4).

In the sparrow, rods occur in similar positions to those in the dark-adapt eye. It is not possible, in the specimens examined, to be sure of the hom logies of the structures observed in the light-adapted sparrow rod, nor is possible to say if movement occurs or not (fig. 4).

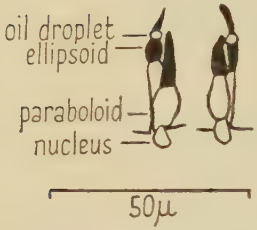


Fig. 5

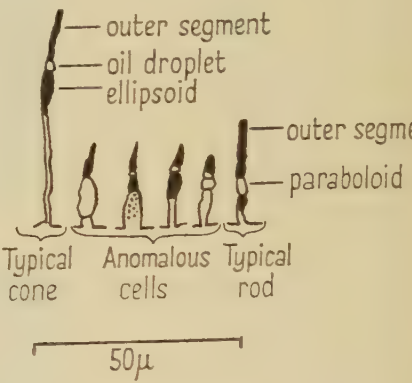


Fig. 6

Fig. 5. Double cones from the periphery of the house sparrow.
Fig. 6. Anomalous cells from the fulmar retina.

Anomalous cells

Structures resembling double cones occur in the sparrow retina (fig. 5 They occupy approximately 30 per cent. of the cells at the periphery and a present to a very small extent in the fundus. Cells similar to the accesso member of double cones were observed on either side of the fulmar pure co region, but none was observed in close union with a cone (fig. 6). They a somewhat intermediate between rod and cone although the oil droplet o served may well be an artifact.

No intermediate cells were found in the shearwater retina. All cells we either distinctly rods or distinctly cones.

A Numerical Comparison of the Visual Cells

Counts of visual cells and ganglion nuclei were made with a $\frac{1}{12}$-inch oil i mersion objective and a $\times 9$ ocular in which a slit diaphragm had been intr duced between eye- and field-lenses so as expose a strip 50 μ in width. Ea section to be counted was orientated under the slit diaphragm so that t visual cells lay parallel to those sides of the diaphragm which were 50 μ apa All the cells in each field were counted at all focus levels; sections were cut 10 μ and each count thus indicates the number of cells observed in a portion retina of area 500 sq. μ.

Tables 1 and 2 show the mean number of visual cells and ganglion nuc which were counted in an area of 500 sq. μ at the regions of the retina indicat

e mean values are calculated from several counts made at each region; the ndard error of the mean (*sx*) and the number of counts made, are also listed. en significance is claimed it is at a 0·01 level of probability.

The house sparrow surpasses both sea-birds in the density of cones at comable parts of the retina, while both sea-birds possess many more rods than sparrow.

BLE 1. *The mean number of rods and cones counted in an area of 500 sq. μ, at the regions of the retina indicated*

gions of etina		Cones			Rods		
		Shear-water	*Fulmar*	*House sparrow*	*Shear-water*	*Fulmar*	*House sparrow*
vea	Mean	37·20	37·80	55·60	Nil	Nil	Nil
	Standard error	1·20	0·69	5·75	..	..	..
	No. of counts	5·00	5·00	5·00	..	..	..
ea cen- alis	Mean	25·67	24·57	41·90	11·67	7·29	Nil
	Standard error	0·84	1·13	0·95	0·41	0·46	..
	No. of counts	6·00	7·00	10·00	6·00	7·00	..
ge of ea cen- alis	Mean	20·28	21·50	25·58	11·54	12·80	4·08
	Standard error	0·98	0·71	0·88	0·60	0·57	0·46
	No. of counts	18·00	10·00	12·00	13·00	10·00	12·00
neral ndus	Mean	10·95	17·80	8·20	11·36	11·40	11·20
	Standard error	0·76	0·72	0·49	0·55	0·46	0·59
	No. of counts	19·00	15·00	5·00	22·00	15·00	5·00

BLE 2. *The mean number of ganglion nuclei counted in an area of 500 sq. μ, at the regions of the retina indicated*

gions of retina		*Shearwater*	*Fulmar*	*House sparrow*
ea centralis	Mean	28·86	26·00	42·40
	Standard error	0·46	1·34	1·21
	No. of counts	7·00	7·00	10·00
ge of area ntralis	Mean	8·89	13·50	22·75
	Standard error	0·72	00·38	00·89
	No. of counts	19·00	10·00	12·00
neral fundus	Mean	2·69	6·80	11·40
	Standard error	0·41	0·67	1·07
	No. of counts	22·00	15·00	5·00

Table 3 shows the internal convergence of visual cells (see definition, p. 348) measured by the ratio of the total number of visual cells to that of ganglion clei in areas of 500 sq. μ. This estimate of the number of visual cells conting with one ganglion cell is approximate only, owing to horizontal conxions in the avian retina, and it does not appear to apply to the fovea of the

sparrow, where the visual cells connect with ganglia situated in the fove ridge (Ramón y Cajal, quoted by Polyak, 1941). However, apart from th fovea, the degree of convergence measured in this way probably correspon fairly closely to the true convergence.

Convergence outside the fovea is greatest in the shearwater and least in th sparrow.

TABLE 3. *The ratio of the total number of visual cells to that of ganglion nucl*

Regions of retina	*Shearwater*	*Fulmar*	*House sparrow*
Area centralis . .	1·3	1·0	1·0
Edge of area centralis	3·6	2·5	1·3
General fundus .	8·3	4·3	1·7

DISCUSSION

The two sea-birds resemble one another and differ from the passerine the structure of the area centralis. In both shearwater and fulmar it is linea and lies below the horizontal axis of the eye, whereas in the sparrow it circular and lies above the axis. This difference may be related to the mode life of the birds. The circular area is frequently found in tree-dwelling form (e.g. hoopoe, humming bird, and red-headed woodpecker), whereas the linea area appears to be typical of birds living in a flat and featureless landscape, (e. great bustard, flamingo, ostrich (Wood, 1917)). Pumphrey (1948) has suggeste that the linear area centralis and fovea appear to be suited to fixation of th horizon and to the preferential increase in sensitivity to vertical movements objects in relation to the horizon.

The sparrow possesses a deep convexiclivate fovea, whereas neither th shearwater nor fulmar show this structure. The deep fovea is usually asso ciated with strongly diurnal vision, as it is most highly developed in hawk swallows, and kingfishers. However, the lack of a fovea does not appe necessarily to be universal in sea-birds, for O'Day (1940) records a deep fov in the albatross *Diomedea c. cauta* Gould.

It is generally accepted that two main types of visual cells occur in mo retinae, and that these cells, the cones and rods, are concerned with daylig and night vision respectively. The basis for this duplicity theory is the morph logical distinctness of the two populations of cells in any one retina. Th theory is supported by other evidence. Where an animal possesses only con or only rods it is invariably found that it is wholly diurnal or wholly nocturna respectively. The nerve connexions to each type of cell are different, ma rods, but only one or a few cones, being connected to one fibre (Polyak, 194 The Purkinje phenomenon further suggests that two distinct populations cells are involved.

The pure cone region of the retinae examined differs in extent in the thr species. This is probably a reflection of the habits of the birds. In the shea water the pure cone region is small in order to minimize the area which

n-functional at night and yet large enough to provide a region of accurate ion for day use. It seems, however, too small to allow the development of a ea (see fig. 2). To some extent this may also be true of the fulmar. The ex- sive pure cone and cone-rich region of the sparrow is in keeping with the ongly diurnal habits of this bird.

Considerable change of the rods in dark- and light-adapted retinae respec- ely has been observed in the shearwater and fulmar but not in the sparrow. rten (1907) showed that movements occurred in the fowl and pigeon, and ey (1915) reviewed all reports of photomechanical changes in the retinae of rtebrates. His conclusion was that while the movements are probably re- ed to conditions of light and darkness, their functional significance remains scure. The placing of rods near the external limiting membrane in the dark- apted eye may have some bearing on sensitivity.

FIG. 7. Schematized sections of three whole eyes; see text for explanation.

The fulmar and shearwater retinae are very similar. The anatomical differ- ces are, in the shearwater, the larger rod paraboloid, the greater number of ds in the area centralis, and the slightly higher ratio of visual cells to ganglion clei.

Owls show a number of adaptations to the nocturnal habit. Sensitivity is hieved largely by the sacrifice of features which give acuity in the diurnal e. In addition to large numbers of filamentous rods and a high ratio of visual lls to ganglion nuclei, the structure of the eye as a whole is modified. The ameter of the lens is increased, it becomes more spherical, and to accom- odate it the anterior portion of the eye is enlarged. At the same time, the sterior portion is reduced in size (Walls, 1942). This study has been con- rned mainly with visual cells, and accurate measurements of the dimensions the eyes of the three species have not been made. However, fig. 7 shows hematized sections of the eye of owl, shearwater, and pigeon.

No extreme specialization of the eye as a whole, as is evident in the owl curs in the shearwater.

Nightjars have large eyes, specialized in much the same manner as those of e owl, but in addition show 'eyeshine', presumably caused by a tapetum Valls, 1942). No 'eyeshine' was observed in any of many shearwaters handled night by torchlight, although this was not specifically looked for.

It seems therefore, that sensitivity in the shearwater is achieved in entirely different manner from that in owls and nightjars. It is, of cour possible that some other sense is used in conjunction with vision. For examp Lockley (1942) suggested that the call is important, in homing to the nest.

Acknowledgements

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Growth and Cellular Proliferation in the Early Rudiments of the Eye and the Lens

By B. I. BALINSKY

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Summary

1. The relation between growth, cellular proliferation, and morphogenetic movements was investigated in the case of lens formation in *Elephantulus myurus jamesoni* and *Xenopus laevis*.

2. For this purpose the volume of the eye cup and lens rudiments was estimated, counts of cells were made, and at the same time counts of cells in mitosis. The mitotic index was calculated, and the material wherever possible was treated statistically.

3. The lens rudiment grows at a greater rate than the eye cup rudiment during the stages in which the lens is being formed. The rate of cellular proliferation in the lens rudiment is also higher than in the eye cup rudiment. The size of the lens cells remains constant whilst the size of the eye cup cells diminishes during the period investigated (at least in *Xenopus*).

4. The mitotic index in the lens material is lower than in the eye cup material. This indicates that the duration of mitosis in relation to the interkinetic period is, in the eye cup rudiment, greater than in the lens rudiment.

5. The mitotic index in the lens material does not increase or decrease significantly during any stage of the lens development, nor were there found any other indications of an increased or decreased growth or proliferation of the lens material. It is therefore concluded that the formation of a visible lens rudiment is due to morphogenetic movement—contraction of a sheet of cells towards the centre of the future eye cup.

It is well known that unequal growth is a major factor in establishing the final proportions of the body in animals. It is not so clear what part unequal growth plays in the earlier phases of morphogenesis, that is, in stages when the organ rudiments are first formed and acquire their general shape. The growth of the embryo has been studied successfully in many animals, but not much has been made known concerning the growth processes in stages when the organ rudiments are being formed. In previous papers I have examined the growth and cellular proliferation in the earliest rudiments of the mammary glands (Balinsky, 1950*a*, 1950*b*). I have now found it desirable to investigate the same processes in organ rudiments of a different type. The organ selected was the lens of the eye throughout the period when it is being formed from cells of the embryonic epidermis. I have also studied the growth of the eye cup in the same stages for comparison.

Material and Methods

The investigation of the growth processes in early rudiments requires a special approach and a special technique. It is impossible to determine the

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size of parts by weighing them after dissection as is done with organs of t older embryos and juvenile animals. The size must be estimated by sor method of reconstruction from serial sections of the embryos. As an addition method of estimating growth, cell counts in serial sections may be used, ar also the estimation of the mitotic index (percentage of cells in mitosis relation to total number of cells) which can reasonably be supposed to be co related with the rate of proliferation in a given tissue. As all these methods a very laborious, only a small number of embryos could be treated in this wa

Accordingly, two series of embryos were made available for this wor The first series comprises five embryos of the elephant shrew, *Elephantul myurus jamesoni*, selected from the embryological collection of Prof. C. J. va der Horst at the Zoological Department of the Witwatersrand Universit The uteri with embryos were preserved in Bouin and cut in serial section In the youngest embryo (25 pairs of somites) there is no trace of the le thickening, in the oldest embryo (40 pairs of somites) the lens is rounded ar completely separated from the epidermis, and its proximal wall is bein thickened, owing to the beginning of the differentiation of the fibres. The was no means of estimating the actual age of the embryos and the time whic is required for the embryo to pass from the 25-somite stage to the 40-somi stage.

The second series was that of embryos of the clawed toad, *Xenopus laevi* specially preserved for the purpose of this work. A number of embryos we kept at room temperature, and groups of embryos were preserved over period of 24 hours. The temperature range was 19° to 25° C. The seri (19 embryos) covers the period from stage 15— to stage 19+ (stages aft Weisz, 1945). The embryos were preserved in Bouin, stained in block wi borax carmine, and embedded for sectioning in celloidin-paraffin wax.

The *Xenopus* series was later supplemented by a small additional one t cover a gap in the original series where the groups of embryos seemed t have been taken too far apart. The embryos of the additional series (9 em bryos), unfortunately, are not in all respects comparable with the main serie as the eggs of this batch turned out to be larger, and correspondingly th eyes and lenses were larger than in comparable stages of the first series.

The morphological changes taking place in the eye rudiment during th stages covered by my investigation are shown in a series of drawings repr senting typical transverse sections through the eye and lens rudiments *Xenopus* (fig. 1).

In stages 15— to 17 there is no thickening of the epidermis to form th lens, and the outer surface of the eye vesicle is uniformly convex. The ep dermis consists of two distinct layers: the outer or covering layer, and th inner or 'sensory' layer. Only the latter is concerned in lens development.

In stage 17+ the outer surface of the eye vesicle begins to be invaginate starting with the upper rim of the future eye cup (fig. 1, D). At the same sp the sensory layer of the epidermis becomes thickened whilst the other par of the future lens rudiment do not seem to be affected.

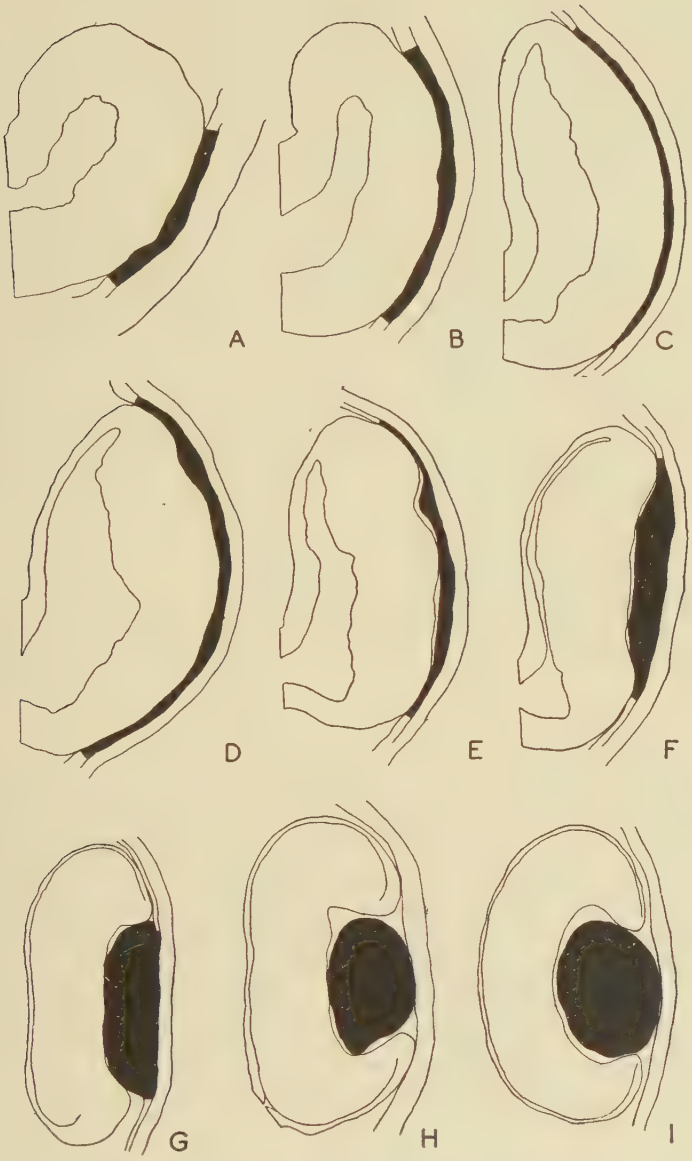

FIG. 1. Typical transverse sections through the eye rudiment in *Xenopus*. A, stage 15—. B, stage 16—. C, stage 17. D, stage 17+. E, stage 17++. F, stage 18—. G, stage 18. H, stage 19—. I, stage 19+.

In stages 17++ and 18— the invagination of the eye cup progresses furth (fig. 1, E, F) and at the same time the portion of epidermis forming the le becomes distinctly thickened throughout its whole area.

In stage 18 I found the lens already a solid mass of cells lying in the openi of the eye cup, but still not separated from the epidermis (fig. 1, G).

In stages 19— and 19+ the lens is completely separated from the epiderm and the fibre differentiation has started.

The above is a description of the development as it could be reconstruct from the more typical specimens. Although the embryos selected for preserv tion in each group seemed to be in the same stage of development as regar the external features, I found later that the degree of development of the e and lens in embryos of the same stage varied considerably. These variatio caused some overlapping between the groups of embryos belonging to diffe ent stages in so far as the eye and lens development were concerned. So as n to bias the results of the investigation, I did not discard the aberrant specime Fortunately, however, I found that there are three distinct natural periods development which could be clearly discerned, and between which there is overlapping. The periods are:

From stage 15— to stage 17.
From stage 17+ to stage 18.
From stage 19— to stage 19+.

I will have occasion to make use of these three periods in my further analy of the data.

I estimated the volumes of the rudiments (eye and lens) as in my previo investigations (Balinsky, loc. cit.) by drawing sections of the rudiment with camera lucida, and then measuring the surface of the drawings with a pla meter. In some cases all the sections were drawn and measured (lens *Xenopus*). In other cases only every second or every third section was draw and the total volume was estimated by 'graphical reconstruction' (Dornfe Slater, and Scheffe, 1942). In all cases the volume was estimated in arbitra units. It would have been possible to calculate the volumes in absolute un from these data, but I did not find this necessary as only the relative increa were of interest in this investigation. Before the formation of the lens thicke ing, that part of the sensory layer of the epidermis (in *Xenopus*) or of the wh epidermis (in *Elephantulus*) with which the outer surface of the eye vesi is in immediate contact, was measured as the equivalent of the later le rudiment.

In the case of the lens rudiment all cells were counted in every section a the number of cells in mitosis was noted separately. As 'mitoses' were coun cells beginning with the metaphase (nuclear membrane dissolved), and endi with the early telophase (contour of the nucleus still irregular). It is inevita that some cells should be counted twice if they are halved between two c secutive sections. The total number of cells counted must therefore be

ess of the actual number of cells present in the rudiment. A uniform pro-
ure of counting was adopted in every case, however, and the relative in-
ases in the number of cells should therefore not be appreciably distorted.
e same applies to the estimation of the mitotic index, as mitotic cells would
sometimes be counted twice in two consecutive sections.

y dividing the volume of the lens rudiment by the number of cells in the
e lens I calculated an 'index of cell size'. Although the index is presumably
portional to the average size of the cell in the rudiment, the cell volume
not be directly calculated in absolute units for both reasons mentioned
ve: (1) because the volume was determined in arbitrary units, and (2) be-
se the cell counts give an exaggerated estimate of the number of cells
sent. In the case of the eye vesicle and eye cup the cells were counted only
selected representative sections. These counts were used for estimation of
mitotic index. Special counts of cells in selected sections have been made,
correlate cell number with volume of the eye rudiment. (This part of the
rk was performed by Miss V. Gubbay.) The sections were drawn as before
h a camera lucida, and the number of cells counted in the section was
ided by the surface of the section. This gave the number of cells per
itrary unit of volume.

In *Elephantulus* I used both eyes of each embryo for measurements and
nts. In *Xenopus* embryos I used only one eye in each (usually the left).
e mitotic index was calculated for each rudiment separately, and I also
culated average mitotic indexes for groups of embryos by pooling the data
all rudiments investigated.

The cell and mitosis counts were treated statistically for the purpose of
ting (1) whether counts made on the same tissue in different embryos
ght be considered as representing one homogeneous population of cells,
d (2) whether there are significant differences between data obtained for
ferent tissues or for the same tissues in embryos of different stages of
velopment. The tests applied were: (1) the χ^2 test of homogeneity (or test
independence) (Snedecor, 1946, pp. 188 ff.); (2) analysis of variance, with
gular transformation of percentages, and partial weighting (after Cochran,
43); (3) in comparing the mitotic index in the eye cup and lens of *Elephan-
lus* I made use of a method suggested to me by Dr. A. Robertson and
ployed by myself in a previous investigation (Balinsky, 1950*a*), that is, the
atment of weighted differences between pairs of counts made in one and
e same embryo.

The Data

(*For Tables 1–3 see pp.* 362, 363, 364).

Analysis of the Data

The first conclusion which can be reached by studying the data presented
this work is that the lens-forming material has a higher rate of growth

TABLE 1. *Volume Measurements (in arbitrary units) and Cell Counts in* Elephantulus

Stage	*Volume, eye*	*Increase by factor*	*Volume, lens*	*Increase by factor*	*Cell counts, eye, mitoses in brackets*	*Eye, mitotic index*	*Cell counts, lens, mitoses in brackets*	*Lens, mitotic index*	*Lens, cell size index*
25 somites	1,020		90		1,357 (41)	3·02	936 (22)	2·35	0·096
	999	1·55	57	2·06	1,249 (57)	4·56	774 (19)	2·45	0·074
27 somites	1,485		54		1,474 (63)	4·27	468 (9)	1·92	0·115
	1,647	1·23	249	2·57	1,455 (48)	3·30	1,901 (38)	2·00	0·131
30 somites	1,953		372		1,270 (36)	2·83	3,294 (81)	2·46	0·113
	1,890	1·95	408	1·90	1,531 (23)	1·50	4,335 (98)	2·26	0·094
34 somites	3,930		696		1,247 (19)	1·52	6,077 (104)	1·71	0·115
	3,576	1·00	783	1·18	1,188 (18)	1·52	6,595 (103)	1·56	0·119
40 somites	3,792		885		1,821 (48)	2·64	7,700 (129)	1·68	0·115
	3,726		867		1,754 (32)	1·82	7,551 (115)	1·52	0·115
Average						2·68		1·81	

Interaction χ^2 for probability of mitosis = 13·33. d.f. = 9. *P* nearly 0·10.

TABLE 2. *Volume Measurements*, Xenopus (*basic series*)

e and time beginning periment	*Eye*			*Lens*		
	Individuals	*Average for stage*	*Increase by factor*	*Individuals*	*Average for stage*	*Increase by factor*
15— hours	4,832 3,264 3,760	3,952		299 80 153	177	
			1·04			1·07
16— hours	4,576 4,224 3,568	4,123		146 265 155	189	
			1·11			1·30
17 hours	5,136 4,016	4,576		352 139	245	
			1·16			1·93
18— hours	4,992 5,616	5,304		535 412	473	
			no increase			no increase
18 hours	5,280 4,016 5,616	4,971		573 344 427	448	
			1·19			1·43
19— hours	6,064 6,128 5,536	5,909		633 767 524	641	
			1·19			1·34
19+ hours	6,784 6,688 7,648	7,040		780 861 932	858	

will be noted that there is no increase either in the size of the lens or of the eye from stage 18— age 18, there is even a slight decrease. This is obviously due to the variability in the size and ée of development of the embryos (overlapping of the adjacent stages).

lume increase) than the material of the eye vesicle and eye cup. This is e in both *Elephantulus* and *Xenopus*.

The total increase is by a factor of:

	Eye	*Lens*
Xenopus . .	1·78	4·85
Elephantulus .	3·72	11·92

The increase of the lens volume is consistently higher than the increase of eye when adjacent stages are compared, though such a comparison is in lf unreliable owing to variability of the specimens. It might have been gested that the relative increase of the lens is not due to an intrinsically her rate of growth, but to an aggregation of cells which are being added he area measured from without (from the surrounding parts of the epi- mis). This cannot apply to the later stages of lens development, after the s rudiment has been segregated from the remainder of the epidermis. The s, however, continues to grow faster than the eye cup even after this has pened (compare stages 19— and 19+ in *Xenopus*).

TABLE 3. *Cell Counts*, Xenopus (*basic and supplementary series*)

Periods	*Stage*	*Eye*		*Lens*		
		Cell counts, mitoses in brackets	*Mitotic index*	*Cell counts, mitoses in brackets*	*Mitotic index*	*Index of cell size*
First period	15—	649 (41)	6·32	143 (5)	3·50	2·09
		352 (19)	5·40	51 (0)	0·00	1·57
		571 (19)	3·33	94 (2)	2·13	1·63
	16—	593 (39)	6·58	108 (0)	0·00	1·35
		688 (2)	0·29	169 (0)	0·00	1·57
		452 (31)	6·86	79 (0)	0·00	1·96
	17	623 (49)	7·67	236 (1)	0·42	1·49
		467 (27)	5·78	135 (3)	2·22	1·03
		855 (64)	7·49	306 (7)	2·29	1·02
		1,043 (55)	5·27	343 (5)	1·46	1·54
Average for period			5·50		1·38	
Second period	17+	1,005 (73)	7·26	355 (4)	1·13	1·84
		936 (67)	7·16	419 (9)	2·15	1·61
	17++	1,064 (46)	4·32	410 (11)	2·68	1·54
		937 (48)	5·12	321 (4)	1·25	1·27
	18—	820 (34)	4·15	409 (9)	2·20	1·70
		860 (44)	5·12	334 (2)	0·60	1·62
		1,019 (61)	5·99	344 (9)	2·62	1·39
		669 (19)	2·84	237 (5)	2·11	2·26
		815 (40)	4·91	208 (3)	1·44	1·98
	18	856 (26)	3·04	338 (11)	3·25	1·70
		850 (57)	6·71	212 (4)	1·87	1·62
		971 (33)	3·40	330 (0)	0·00	1·29
Average for period			5·07		1·81	
Third period	19—	1,225 (48)	3·92	389 (11)	2·83	1·63
		1,080 (27)	2·50	428 (9)	2·10	1·79
		1,038 (38)	3·66	373 (5)	1·34	1·40
	19+	832 (14)	1·68	570 (7)	1·23	1·37
		1,041 (32)	3·07	665 (8)	1·20	1·29
		953 (23)	2·41	627 (7)	1·12	1·49
Average for period			2·95		1·54	

Interaction χ^2 for the whole material . . 40·91. d.f. 27. *P* nearly 0·05.
for the first period . . . 14·93. d.f. 9. *P* nearly 0·10.
for the second period . . 19·24. d.f. 11. *P* nearly 0·05.
for the third period . . . 6.85. d.f. 5. *P* between 0·30 and 0·20.

When analysing the data of the mitotic counts we are confronted with a ver considerable variability in these counts. The test of homogeneity in the cas of *Xenopus* lens cells (Table 3) shows that there is a significant interaction i the probability of mitosis. It is likely, therefore, that the samples are no drawn from a homogeneous population. The variability, however, may com from at least three different sources:

(1) Sampling errors due to the finite numbers of cells counted. In the cas of frequencies this sampling error has the form of binomial variabilit

2) The fluctuations in true mitotic frequency due to differences between the embryos in one and the same phase of development.
3) The variability due to the stage of development of the embryos.

e latter two sources of variability cause 'extraneous variation' in terms used Cochran (1943). Extraneous variation is not dependent on the numbers of s counted. For the purposes of this investigation it was necessary to ferentiate between the three sources of variability. Cochran (loc. cit.) gives ethod for evaluating the proportions of binomial and extraneous variation the total variation. Applying his method I found that binomial variation stitutes the following percentage of total variation:

If equal weights are used . . . . 68 per cent.
If weights proportional to n are used . . 72 per cent.

Analysis of variance may be further applied for differentiating between ividual variation and group variation. In the case where about 70 per cent. variation is binomial and nearly 30 per cent. is extraneous, and where the rcentages studied are based on unequal numbers of counts, Cochran (loc. .) recommends performing the analysis of variance with partial weighting, fixing an upper limit for weights, applicable to two-thirds of the total mber of samples. The results are shown below.

alysis of Variance of Mitotic Frequencies in the Lens of Xenopus *in the three Periods of Development*

	d.f.	*Sum of squares*	*Mean square*
dividuals within periods. .	25	46,516	1860·64
riod means	2	7,181	3590·50
Total	27	53,697	

Variance ratio (F) = 1·9297. For $n_1 = 2$ and $n_2 = 25$, $F_{.05} = 3{\cdot}38$, $P > 0{\cdot}05$.

In the above analysis the variation of the individual embryos contains both mpling variation and 'extraneous variation'. It is obvious that there is no nificant difference of mitotic frequency in the lens material throughout the ree periods of development. I also performed the analysis of variance for the ne stages used with essentially the same result.

The results of the homogeneity test applied to mitosis counts in *Elephantulus* e presented in Table 1. The interaction in this case falls short of the 5 per nt. probability limit. Therefore the assumption that all counts represent one pulation need not be rejected. These results are thus in agreement with the nclusion drawn from the data on *Xenopus*. This is an important conclusion cause it allows us to make the inference that the formation of the lens diment is not connected with significant modifications of the mitotic ivity in the lens material.

In the case of *Xenopus* the mitotic frequency in the eye cup material r mains without significant change throughout periods 1 and 2 (i.e. whi the eye vesicle is being transformed into an eye cup), but there is a sha decline in the frequency of mitoses in period 3. The difference between perio 2 and 3 may be shown to be fully significant. The figures in *Elephantulus* al show a decrease in the percentage of mitoses in the older embryos.

Between the percentages of mitoses in the lens material and the eye c material there is, in *Xenopus*, a very significant difference, the figures for t eye cup being very much higher than those for the lens. When comparing t mitotic index for the eye cup and the lens in *Elephantulus*, one finds that t preponderance for the eye cup is by no means so clear. The average mito index of the eye cup is higher than in the lens (2·68 and 1·81 respectively) b in some counts the relations are reversed. Analysing the difference betwe the mitotic index of the eye cup and the lens by the Robertson method (p. 36 I found that the mean weighted difference is 0·4731, standard error of t mean is 0·2853, $t = 1{\cdot}65$, with 9 degrees of freedom P_t lies between 0 and 0·1. The existence of a difference between the mitotic frequency of t eye cup and the lens is not proved. The ratio between the mitotic frequen of the eye cup and the lens is, however, not stable, as is shown by the presen of a highly significant interaction: P for interaction $\chi^2 = 0{\cdot}01$. This must mainly due to the variability of the mitotic frequency in the eye cup, since t counts for the lens have not shown a significant departure from homogeneit

Some of the data presented in this paper can be used to analyse the relatio ship between the proliferation of cells (increase of cell numbers) and t growth (increase of volume) of tissues. Both should be equal if the volu of each cell is doubled between two successive cell divisions. This, howeve need not necessarily be the case. Tables 1 and 3 show the values of the 'ind of cell size' for lens cells in *Elephantulus* and *Xenopus*. The figures show th the lens cells do not appreciably change in size as development proceeds. T test the significance of the figures, I have applied the analysis of variance to t data for *Xenopus*. I grouped the data according to stage of development fro 15— to 19+, and made the assumption that all subsamples represent o population.

Analysis of variance of the 'index of cell size' in Xenopus

	d.f.	*Sum of squares*	*Mean square*
Individuals within stages . .	19	1·30	0·068
Stage means	8	0·93	0·116
Total	27	2·23	

Variance ratio (F) = 1·70. For $n_1 = 8$ and $n_2 = 19$, $F_{.05} = 2{\cdot}48$, $P > 0{\cdot}05$.

The variance ratio falls short of the 0·05 level. This justifies the assumpti that the cell volume of the lens material does not change significantly duri the period investigated.

n the case of the eye cup the results are quite different. An estimate of the size made by the method described on p. 361 showed that the average cell in stage 19+ is only 0·55 of the cell size in stage 15—. The proliferation of s proceeds with a higher rate than the rate of volume increase. Between ge 15— and stage 19+ the total volume increases 1·78 times, but the rease in cell numbers, calculated from the above data, is by a factor of 3·26. can now discuss the actual significance of the mitotic index as an indicator cell proliferation in tissues. In the *Xenopus* series the embryos were pre-ved at known time-intervals, and the actual increase of the cell number ing these time intervals has been estimated. It is therefore possible to culate what actual rates of increase of cell numbers correspond to certain ues of the mitotic index. I have calculated how often, on the average, each should divide between stage 15— and stage 19+ to account for the in-ase in the number of cells in the lens rudiment and the eye cup rudiment. e formula used for this calculation is the following:

$$\frac{N_2}{N_1} = 2^{(t_1-t_2)/T} \quad \text{(Woodard 1948)}$$

ere N_1 and N_2 are the numbers of cells at the beginning and at the end of period, t_1 and t_2 is the corresponding time, and T is the average interval ween two consecutive cell divisions. The actual time interval (t_2-t_1), it l be remembered, was 24 hours.

The average interval between two cell divisions calculated from the above mula is:

In the lens material . . .	8 hours and 54 minutes.
In the eye cup material . . .	14 hours and 5 minutes.

These data should be compared with the average mitotic index, which is er the whole period 1·63 for the lens and 4·62 for the eye cup. It is obvious t the higher mitotic index for the eye cup does not indicate a higher rate cellular proliferation. The reverse is true of the lens material. This can only an that the duration of the actual mitosis in relation to the interkinetic iod (interphase) is, in the eye cup cells, greater than in the lens cells.

It has been suggested by Woodard (1948) that the mitotic index is not a able indication of the rate of proliferation in tissues. Woodard found t in the neural tube of the 18–72-hour chick embryo the rate of increase of l numbers remains constant whilst the mitotic index decreases. My data not directly confirm Woodard's results, as the discrepancies between the otic index and the rate of proliferation have been found in comparing ferent tissues, and not the same tissue in successive stages of development. is conceivable that in different tissues, having a different mechanism of wth, the relation between mitosis and interphase need not always be the ne, and this of course would greatly change the actual value of the mitotic ex as an indicator of cell proliferation. On the other hand, in parts of the ne tissue, or in tissues having the same type of differentiation and the same

mechanism of growth, the duration of mitosis may remain unchanged, and this case the mitotic index would still be valid as an indicator of proliferatio It is conceivable, however, that the mechanism of growth may change wi incipient differentiation, and perhaps change quickly. It seems obvious th there is still much to be learned about the relation between the mitotic ra and growth, and further investigations in this field are very desirable.

In recent investigations P. Weiss and McKeehan (Weiss, 1950; McKeeha 1951), found that the formation of the lens rudiment is due to change shape of the cells of the future lens which orient themselves perpendicu larly to the surface of the eye cup rudiment, and become columnar instea of flat. This conclusion is supported by my own observations in so f as (1) I could not discover any trace of an increased proliferation of cel during the period when the lens rudiment is being formed; (2) I could n discover any increase in the volume of cells during the period when, accordin to Weiss, they become columnar. I have also found no reason to suspect th additional cellular material is attracted to the site of lens formation: any in crease in the size of the lens rudiment can be accounted for by the rate growth intrinsic to the cells originally situated between the eye vesicle an the outer layer of the epidermis. The actual formation of the lens is carrie out by means of a concentration of its cellular material, i.e. by a morphogeneti movement in the sense of W. Vogt: the diameter of the lens when it is forme is considerably smaller than the diameter of the sheet of cells which are use for its development (see fig. 1, especially the drawings of stages 18—, 18, 19—

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I am greatly indebted to the late Prof. C. J. van der Horst for putting at m disposal his collection of *Elephantulus* embryos. I am obliged to Miss L Dimovic and Miss V. Gubbay for making some of the measurements an counts used in this investigation. I am grateful to Mr. J. E. Kerrich for co sultations on the application of statistical methods in my work. My thank are also due to Dr. D. J. Nolte for helpful advice and criticism.

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A New Type of Light Source for Phase-Microscopy

By D. A. KEMPSON

(*From the Bureau of Animal Population, University of Oxford*)

SUMMARY

special electric bulb is described, with the filament in the form of an exact circle. rovides a very narrow annulus for phase-microscopy without any masking, and gives strong illumination without much heat. Increased contrast is obtained by use of this method.

HE method described here is a modification of the system of phase- microscopy described by Kempson, Thomas, and Baker (1948) and er, Kempson, and Brunet (1949). In this system the phase-plate is placed *nd* (*above*) the rear focal plane of the objective. A large illuminating annulus laced immediately in front of the microscope lamp, and an image of it is de to coincide with the phase-plate annulus by lowering the substage denser and adjusting the mirror.

n this method, and also in ordinary phase-microscopy, a very large pro- tion of the area of the light-source is masked out, only a narrow annulus ng left for illumination of the field of view. The intensity of the illumination consequently much less than in ordinary microscopy. It is still further uced if the illuminating annulus is made very narrow in order to gain reased contrast in the phase-image. Hitherto, the remedy has been to rease the initial intensity of the light by means of a semi-overrun bulb, this generates excessive heat, which is objectionable close to the micro- pe.

The new light-source described in the present paper does not suffer from se disadvantages. It consists of a specially made electric bulb, working at olts, 4 amps. It is similar in size and shape to a motor-car headlamp bulb. e filament is a single-strand wire, formed in the shape of an exact circle of ut 15 mm. diameter. In order to present a complete ring without a break, end of the filament is carried round so as to overlap slightly behind the er end, thus forming rather more than a complete circle. Undesirable ections from the glass envelope are cut off by masking with a suitable le of black paper stuck on to the glass. If a biconvex lens of about 10–15 cm. us is placed in front of the bulb and adjusted axially, a convenient variation apparent size is obtained for use with objectives of different power. The ulus of the phase-plate is made narrower than usual, in order to correspond h the very narrow source of light. As a result, centring of the image of the ment is slightly more difficult than when an ordinary illuminating annulus

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is used, but practice soon overcomes this. It is important that an achromat substage condenser should be used to throw a good image of the filamen

The method gives good images and strong contrast.

Dr. John R. Baker has contributed largely to the development of th illuminant, for which the author is duly grateful. The research was financ out of a grant made to Dr. Baker by the Parliamentary Grants Committee the Royal Society. The special electric bulbs were made by Mr. Dougl Saxton of the Clarendon Laboratory, Oxford.

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Mounting Collodion Sections before Staining

By K. C. RICHARDSON

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Summary

ollodion sections arranged on an albumenized slide or sheet of mica containing a r of turpentine are covered with a thin sheet of cellophane previously soaked in er. The sections remain visible so that the folds can be smoothed out. Then the paration is covered again with a slide or a strip of metal and placed under pressure 5° C. After a short interval the cellophane can be stripped off and the collodion loved completely. When the preparation is treated like a mounted paraffin section r removal of wax, the attachment is firm, and protection from loosening by ering with a thin film of collodion is unnecessary.

N the standard textbooks on microscopical technique many ways are quoted for attaching collodion sections to slides before staining. With some of these thods the attachment is so precarious that extra care is needed during the nipulations of staining and dehydration: with others the section is covered h a film of collodion or stuck to a layer of gelatine. Such supporting films, wever, often take up some of the stain. The method to be described is mple and reliable and can be applied to all types of collodion technique, ging from thin sections cut in collodion-paraffin or low viscosity nitro-llulose, to the large, thick sections commonly cut from collodion blocks rdened by evaporation or chloroform and stored in alcohol. In each case it is ssible to remove the collodion immediately after mounting so that the tion becomes comparable with a routine paraffin section, and will with-nd prolonged treatment even in hot solutions without becoming detached.

Mounting on Glass Slides

1. Smear a clean slide with Mayer's albumen-glycerine, using about the ne quantity as for mounting paraffin sections.

2. Add a drop of pure turpentine over the area to receive each section and nsfer the sections, previously stored on thin paper soaked with 70 per cent. anol, so that the collodion lies as flat as possible on the albumenized surface. necessary add a few drops of turpentine to each section to ensure that they e evenly soaked, but avoid floating the sections out of alinement.

3. Cover the sections carefully with a strip of thin cellophane (25–50μ ck), previously cut to the same size as the slide, and soaked in water until np. It is unnecessary to remove the surplus water beyond simple draining fore placing the cellophane in position.

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4. Lift up the slide, holding one end of the cellophane and glass firmly, a stroke the cellophane lightly in several directions with a finger or a brush that the excess turpentine drains off and the folds in the section begin smooth out. Increase the pressure of the finger as the sections become flatte

5. Now polish the sections quite flat with a duster. Considerable pressu may be used without splitting the cellophane or injuring the section. Duri this stage it is essential to hold the cellophane firmly in position with t thumb and forefinger, and to be careful that the cellophane does not becon too dry or it will tend to recoil from the sections, which will then dry o almost instantaneously.

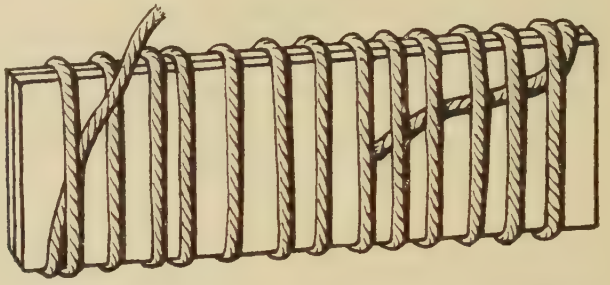

Fig. 1. Method of tying with string before placing the mounted collodion section in the incubator.

6. Cover immediately with a strip of thin paper moistened with turpentin Place a clean slide on top of the paper and bind the 'sandwich' tightly wit string (see fig. 1).

7. Place in an incubator at 55° C. for ½–1 hour. The albumen sets, but th section does not completely dry out at this temperature if it is well boun together. It is important, however, to remember that once the 'sandwich' untied, drying will take place quite rapidly and the cellophane will recoil not held in position.

8. Cautiously remove the top slide, strip off the paper, but hold the cell phane in contact until it can be made wet and limp again. This can be done b holding the preparation under a hot-water tap for about 10 sec., or by plungin the slide with the section and cellophane undisturbed into a dish of war water.

9. Drain off the excess water, peel off the cellophane, and quickly plun the slide into a dish of xylene.

10. Transfer through several changes of acetone until the collodion dissolved completely.

11. Transfer to absolute ethanol, and continue the staining procedure for a paraffin section.

Mounting on Mica

1. Clean a sheet of mica (e.g. 7·5 × 5 cm.) in acid alcohol (70 per cen ethanol + 0·5 per cent. HCl). Wash in water and place the wet mica on a she

zinc (0·5 mm. thick) cut to slightly larger dimensions than the mica.
ish the mica dry with a clean duster.
. Continue with stages 2–5 as above.
. Cover immediately with a strip of thin paper moistened with turpentine.
ce a second sheet of zinc on top.
. Place the 'sandwich' on a sheet of thick cardboard wrapped in several
ers of paper or on a sheet of thick rubber, and cover with a domestic iron
ted to about 55° C., which is held in position under pressure with a weight
l lever arrangement as shown in fig. 2.

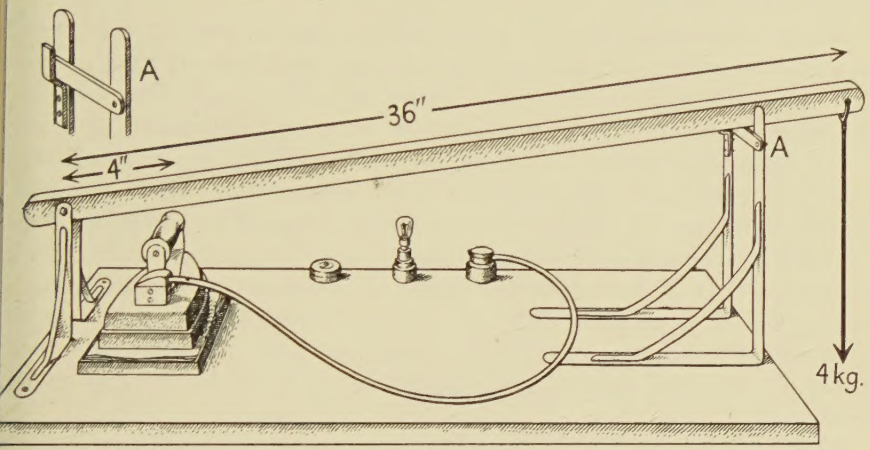

FIG. 2. Hot-press made from four shelf brackets and a wooden lever. When the cross-bar at s disengaged the lever, to which a weight of 4 kg. is attached, is lowered gently to press on handle of the iron at a point about 4–5 in. from the fulcrum.

5. After 10 min. heating continue stages 8–11 as above.
6. On completing staining and dehydration the mica sheet is placed from lene on to a sheet of paper already soaked in xylene. By cutting with scissors rough both mica and paper together the small pieces of mica bearing the ctions are isolated without fracturing. Each piece is then pressed down on o of a small drop of mounting medium placed on a clean slide. Then further edium is added to cover the section, and the coverglass lowered into position.

DISCUSSION

Methods resembling the above have been described by Bensley and Bensley 938) and by Baker (1950). It is claimed that the present technique is simpler d quicker to operate, and the introduction of cellophane makes the flattening the section easier to control because it is transparent and more robust than t cigarette or filter paper. It should be emphasized that with all these ethods much depends on the way the section is cut, particularly if it is thin. blunt knife or insufficient infiltration of the block will cause the material to etch so that the section comes off the knife in the form of a relatively extensible frame of collodion surrounding a dome-shaped slice of tissue.

When flattened in this condition the material must inevitably fold. Successf sticking of the section depends upon firm pressure being maintained duri heating, and this is particularly necessary for dense tissues such as bone cartilage. Various types of spring clips and weights placed on the slide in t incubator have been tried, but the method of tying with string has been fou to be less cumbersome when large numbers of sections are being mounte and rarely cracks the slides. To quote an extreme example of the usefulness the method, the author has found it possible to mount on photographic hal plates collodion sections 100μ thick cut horizontally through half a goat udder in full lactation.

The second method, involving the use of an electric iron, must be regarde as a rapid procedure suitable for sets of routine class sections which are to b cut, mounted, and stained in the course of a few hours. Occasionally th severe pressure of the iron will produce areas of compression in a sectio which stain as light patches with strong dye combinations such as Mallory phosphotungstic acid haematoxylin. With routine stains these artifacts a invisible and do not detract from the great advantage which the metho provides in speeding up the preparation of large class series of material whic must be cut in collodion. When mounting large numbers of small sections on mica sheet or large glass slide it is better to add the turpentine in sma quantities for each row of sections. A large pool of turpentine covering th entire albumenized surface makes it difficult to keep the sections correctl orientated.

The unconventional steps of mixing water with turpentine in stage 6, an water with xylene in stage 9 are indispensable for smoothing the cellophane an preventing it sticking to the sections. No interference with subsequent stai ing reactions seems to result from these mixtures. The removal of collodio by acetone rather than alcohol-ether is preferred because the latter solve swells the collodion before dissolving it, thus weakening the attachment of th sections. Strong alkalis like ammonia used at any stage after mounting will, course, loosen paraffin or collodion sections attached with egg albumen.

Although not strictly relevant to the mounting of sections there are tw procedures for cutting with a sliding microtome which might be mentioned i conclusion. Firstly, the addition of a trace of photographic wetting agent (e.g No. 326 wetting agent, Johnsons of Hendon Ltd.) facilitates even spreadin of 70 per cent. ethanol on the blade of the knife, and does not interfere wit mounting or staining. Secondly, a narrow strip of thin mica placed under th knife can be used as a spatula in preference to forceps for picking up very thi collodion sections. The sections are floated with excess alcohol from the knif to the mica, and then from the mica direct to the slide. This avoids collapse c severe folding.

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